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AMPHETAMINE SULFATE-ETHYL ALCOHOL ANTAGONISM IN THE RABBIT*

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DURING an investigation in man of the stimulating action of amphetamine (benzedrine) sulfate on abnormal mental states characterized by depression, certain toxic depressive states caused by alcohol were included, and it was observed that the alcoholic cases were among those most favorably affected.[1-4] These observations indicated that in man there is an antagonism between amphetamine sulfate and ethyl alcohol.

In a series of over 100 cases of alcoholism, with and without psychosis, our most prominent results of treatment included: (1) a reduction by one-half in the length of time for recovery of the acute alcoholic psychoses; (2) a slight increase in the number of recoveries; (3) a diminution in the severity of the psychotic manifestations; (4) a dissipation of the "hang-over" effects of inebriation; (5) an unsatisfactory response in chronic alcoholic addiction; (6) a differentiation between uncomplicated alcoholic depressions and psychogenic depressions masked with alcohol by the rapid response of the former; and (7) an antagonistic or "sobering" effect of amphetamine during acute alcoholic intoxication. The latter was most striking. Boisterous, excited, hyperactive, surly and irritable individuals became quieted; some fell asleep after the medication. Tremor in these patients was aborted. Occasionally, however, the excitement and the tremulousness was increased. The incoherence and incoordination of more profound inebriation was rapidly replaced by a more coordinate state. Persons who had imbibed sufficiently to become stuporous frequently were aroused and rational within thirty minutes following the intravenous injection

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of 20 to 30 mg. of amphetamine sulfate. The drug rendered depressed, sullen, and asocial intoxicated individuals more cheerful and adaptable. Some chronic alcoholic patients took amphetamine before drinking in order to consume larger quantities of alcohol without the appearance of unpleasant symptoms. We concluded from these investigations that in man there is an antagonism between amphetamine sulfate and alcohol, which is of greatest value clinically in the more acute phases of alcoholism with and without psychosis.

PRESENT INVESTIGATION

Because of the satisfactory response of acutely alcoholic patients to amphetamine sulfate, it seemed desirable to test in animals the limits of the antagonism between the two drugs. Since this investigation was begun (October, 1938), Werner[5] has published a brief report on the effects of amphetamine sulfate on alcoholic depression in animals. He stated that 7 c.c. per kilogram of alcohol orally protected rabbits against the lethal effects of subsequently administered amphetamine sulfate. The lethal dose of amphetamine was increased approximately 1.5 times. The drug did not completely awaken the depressed animals. However, in rabbits receiving 2.5 c.c. per kilogram of alcohol intravenously, coordination of the hind legs was restored by amphetamine. Entirely similar results were obtained by Werner with pyridine-B-carboxylic acid diethylamide (coramine), metrazol, and picrotoxin.

MATERIALS AND METHODS

Inasmuch as both the alcohol and the amphetamine sulfate had been administered orally in the clinical studies, it was decided to administer the drugs to animals by the same route. The rabbit was used because of suitable size for ease in passing a stomach tube. From a colony of about 800 rabbits, approximately 600 were chosen at random and employed in the present investigation. These animals were equally distributed in sex, and varied in age from 6 to 15 months, and in weight from 1.50 to 3.20 kg. Subsequently, the experimental group was reduced to 423 by eliminating from the data those rabbits weighing less than 1.70 or more than 3.00 kg., because of the observation of Ehrich, Lewy, and Krumbhaar[6] that the minimum lethal dose varied with the weight (age) of the animals and that the heavier animals were more susceptible. However, in no instance were the results modified by thus restricting the number of animals. No seasonal variation in the susceptibility of the animals to the same mixture of the drugs was observed. In many instances the solutions containing different mixtures were employed simultaneously, thus rendering the variations in response more easily comparable.

All animals were fed on a standard laboratory diet, which had maintained in good health for several years the remaining 200 rabbits (employed in pregnancy tests). In every experiment food was withheld routinely for more than sixteen hours before and after medication. No animal was used more than once throughout the investigation. All medications were administered intragastrically as solution, using an 18 F urethral catheter as an esophageal tube. Preliminary experiments indicated that the average stomach capacity of the selected animals was 150 to 250 c.c. All solutions were devised, therefore, so

that the total volume of a single dose never exceeded 150 to 175 c.c. (depending on the size of the animal). All solutions were made, however, so that the volume administered would be as large as possible, in order to minimize drug loss and in order to eliminate the necessity of washing down a given dose with additional fluid and thus changing the concentration. To secure uniform freshness, the solutions were prepared as needed. The amphetamine sulfate used was the racemic compound, in the form of a powder,* dissolved in distilled water. The alcohol solutions were prepared from alcohol U.S.P. (i.e., 95 per cent).

The particular techniques are described in connection with the divisions of the problem. In the lethal dose experiments the dose was considered to be fatal only if the animal died within fourteen days of medication. The term "minimum lethal dose $_{50}$ (M.L.D. $_{50}$)" is used to describe that dose which was lethal to at least 50 per cent of the animals to whom it was given.

TABLE I

ANALEPTIC EFFECT OF AMPHETAMINE SULFATE ON NARCOSIS OF MODERATE AMOUNTS OF ETHYL ALCOHOL

CONTROL GROUP 5 GM. ALCOHOL PER KG.			TEST GROUP 5 GM. ALCOHOL PER KG. AND 85 MG. AMPHETAMINE PER KG.		
ANIMAL NUMBER	ONSET OF NARCOSIS IN MINUTES	DURATION OF NARCOSIS IN MINUTES	ANIMAL NUMBER	ONSET OF NARCOSIS IN MINUTES	DURATION OF NARCOSIS IN MINUTES
F3	27	293	F25	77	275
F6	10	340	F26	57	353
F9	20	290	F28	54	320
F12	17	520	F29	45	280
F13	15	425	F30	81	257
F15	23	485	F31	69	251
F17	26	354	F32	32	345
F19	14	463	F34	41	345
F20	15	344	F35	30	294
F21	12	345	F36	57	264
F22	8	479	F37	53	295
F23	18	461	F38	57	363
F24	22	505	F40	74	349
Total	227	5304		727	3991
Average	17.4	408		55.9	307

RESULTS

A. *Moderate Dose Experiments.*—The following experiments were performed to determine the effect of amphetamine sulfate on the narcosis of moderate amounts of ethyl alcohol (Table I):

(1) To a series of 13 animals (control group), 5 Gm. per kilogram of alcohol were administered intragastrically. The onset and the duration of narcosis were recorded. The animals were stimulated at five- to ten-minute intervals by a forceful pinching of the ear with a mouse-toothed forceps, and were not considered to be in a state of narcosis if they could coordinate their hind legs sufficiently to hop once without losing balance. Narcosis occurred in 17.4 minutes (average) after the administration of the alcohol, and lasted 408 minutes (average).

*Racemic amphetamine sulfate powder was supplied through the courtesy of Mr. Theodore B. Wallace, Smith, Kline and French Laboratories, Philadelphia, Pa.

(2) The same experiment was performed in another series of 13 animals (test group) to whom 5 Gm. per kilogram of alcohol and 85 mg. per kilogram of amphetamine sulfate were given in the usual manner. Sleep ensued in 55.9 minutes (average) and lasted 307 minutes (average).

Comparison reveals that the addition of the amphetamine sulfate delayed the onset of the narcosis about 200 per cent, and shortened it about 25 per cent.

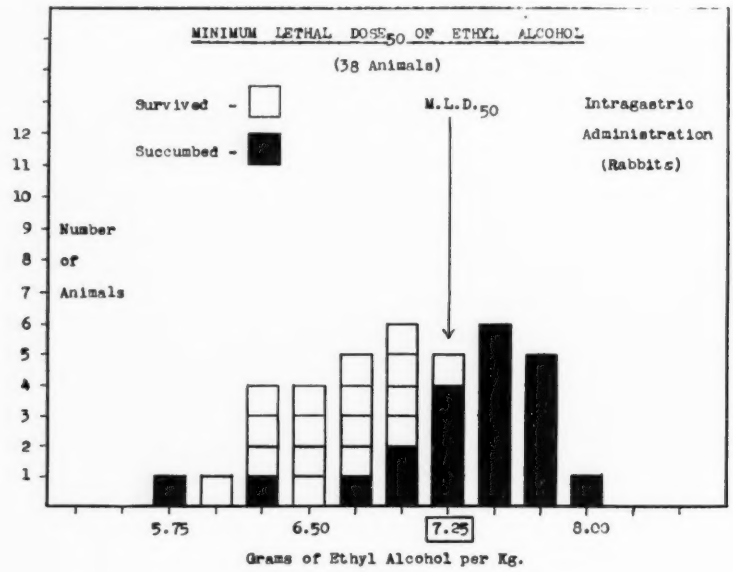

Fig. 1.

Statistical Treatment of Results: Onset of narcosis—the standard error of the mean of the control group was 1.786 minutes and of the test group, 4.94 minutes. The ratio of the difference of the means to the standard error of their difference was 1.857, indicating but thirty-two chances in one thousand of a reversal in similar experiments. *Duration of narcosis*—the standard error of the mean of the control group was 7.869 minutes and of the test group, 12.9 minutes. The ratio of the difference of the means to the standard error of their difference was 11.5, indicating an infinitesimal chance that there would be a reversal in similar experiments. The degree of significance of the changes of the onset and the duration of the narcosis is increased by the fact that amphetamine sulfate altered both of these factors in the same direction.

B. *Lethal Dose Experiments.*—Five experiments were performed to determine the effect of amphetamine sulfate on lethal doses of ethyl alcohol, and the effect of ethyl alcohol on lethal doses of amphetamine sulfate:

(1) *M.L.D.* $_{50}$ *of Ethyl Alcohol:* Varying doses of 25 per cent ethyl alcohol solution were given to 38 animals, and the number of deaths within fourteen days was recorded. The volume of the solution administered varied from 58 to 112 c.c. The M.L.D. $_{50}$ was found to be 7.25 Gm. of alcohol per kilogram (Fig. 1).

(2) *M.L.D.* $_{50}$ *of Amphetamine Sulfate:* In a manner similar to the preceding experiment, 0.25 per cent solution of amphetamine sulfate was given to 68 animals. The volume of the solution administered varied from 44 to 130 c.c. The M.L.D. $_{50}$ was found to be 85 mg. per kilogram (Fig. 2).

(3) *M.L.D.* $_{50}$ *of Mixture A:* Mixture A (7.25 Gm. of ethyl alcohol (1 M.L.D. $_{50}$) and 42.5 mg. of amphetamine sulfate (½ M.L.D. $_{50}$) in 37 c.c.) contained alcohol and amphetamine in approximately 25 per cent and 0.15 per

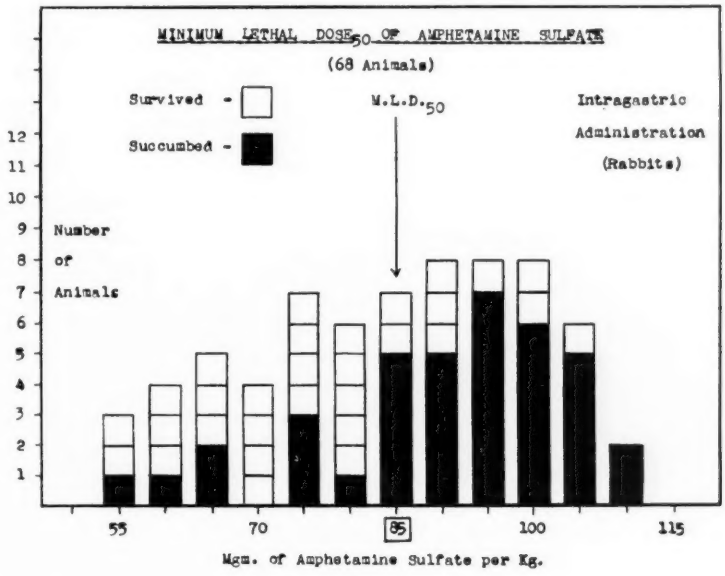

Fig. 2.

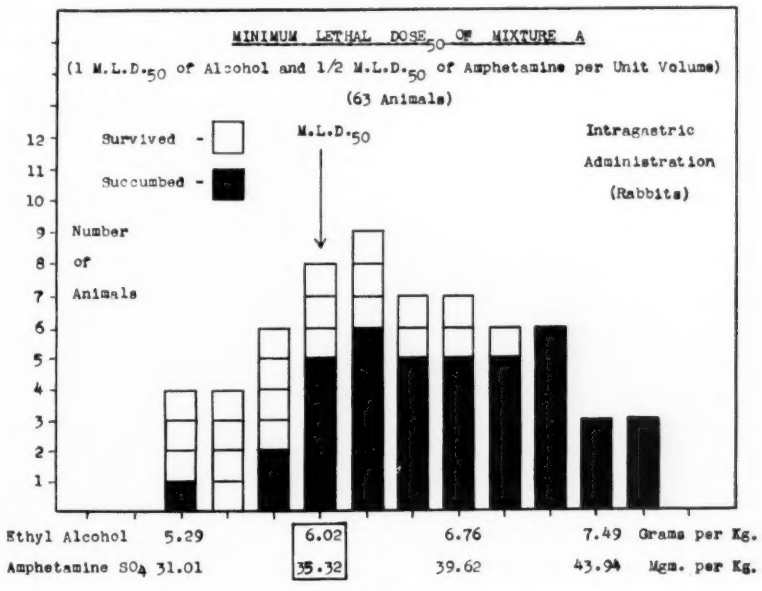

Fig. 3.

cent concentration, respectively. Varying quantities of this mixture were administered to rabbits, and the number of deaths within fourteen days was tabulated. The amounts of ethyl alcohol per kilogram and amphetamine sulfate per kilogram for a given quantity of mixture A solution were then calculated.

The volume administered varied from 45 to 115 c.c. A series of 63 animals was required. The M.L.D. $_{50}$ of the mixture A solution was found to contain 6.025 Gm. per kilogram of ethyl alcohol and 35.32 mg. per kilogram of amphetamine sulfate (Fig. 3).

(4) *M.L.D.* $_{50}$ *of Mixture B:* The procedure was the same as the preceding one. Mixture B (7.25 Gm. of ethyl alcohol (1 M.L.D. $_{50}$) and 85 mg. of amphetamine sulfate (1 M.L.D. $_{50}$) in 37 c.c.) contained alcohol in approximately 25 per cent solution, and amphetamine in approximately 0.3 per cent solution. A volume of solution varying from 47 to 102 c.c. was given to 55 animals. The M.L.D. $_{50}$ of the mixture B contained 5.78 Gm. per kilogram of ethyl alcohol and 67.72 mg. per kilogram of amphetamine sulfate (Fig. 4).

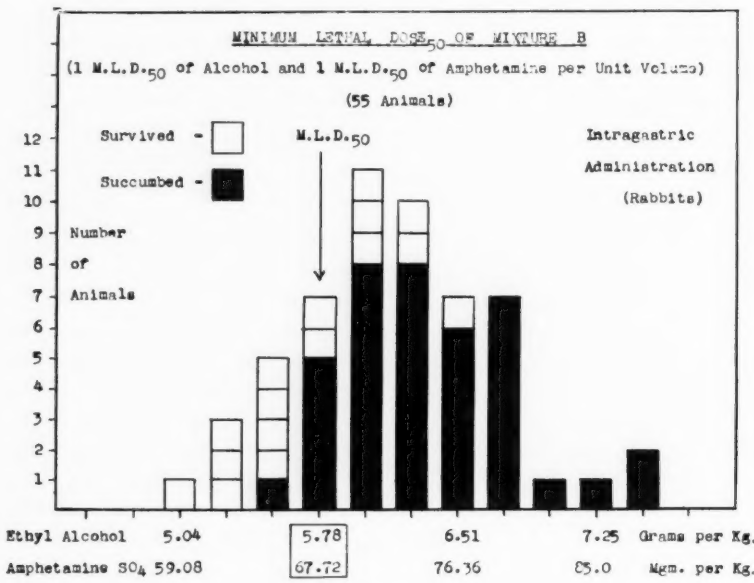

Fig. 4.

(5) *M.L.D.* $_{50}$ *of Mixture C:* The same technique was employed. Mixture C (3.625 Gm. of ethyl alcohol ($\frac{1}{2}$ M.L.D. $_{50}$) and 85 mg. of amphetamine sulfate (1 M.L.D. $_{50}$) in 34 c.c.) contained alcohol in approximately 16 per cent solution, and amphetamine in approximately 0.25 per cent solution. A volume of solution varying from 62 to 175 c.c. was administered to 99 animals. The M.L.D. $_{50}$ of the mixture C solution was found to contain 5.239 to 5.968 Gm. per kilogram of ethyl alcohol and 125 to 140 mg. per kilogram of amphetamine sulfate (Fig. 5). It should be noted that even in this large series of animals, there was so much individual variation that an exact single value for the M.L.D. $_{50}$ could not be determined. Inasmuch as the range of values obtained was satisfactory to indicate the trend, the experiments were terminated.

ANALYSIS OF RESULTS AND DISCUSSION

The results of animal experimentation cannot be strictly applied to man. However, in the experiments herein recorded, amphetamine sulfate has exhibited

an antagonistic effect in rabbits intoxicated with moderate quantities of alcohol, and this seems to confirm the clinical observations previously cited of the effectiveness of amphetamine sulfate in acute alcoholic states in man.

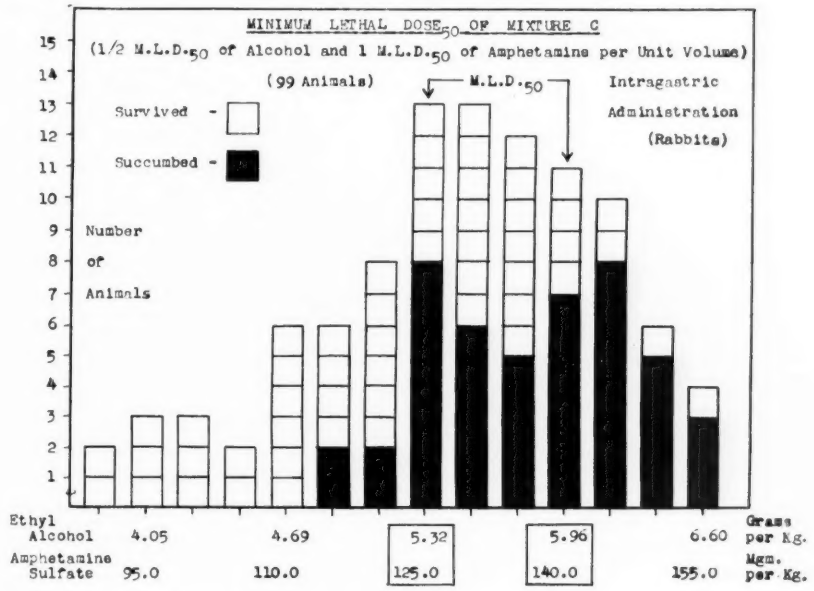

Fig. 5.

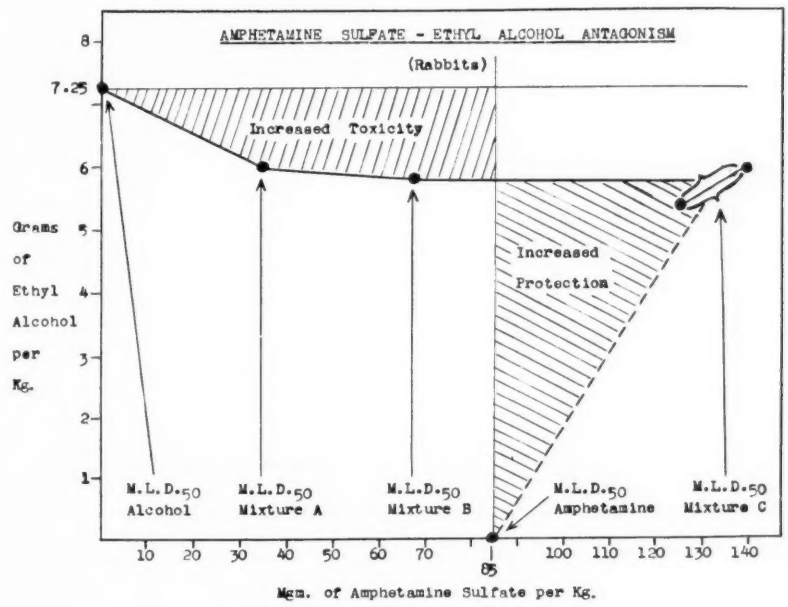

Fig. 6.

In the lethal dose experiments, however, the addition of amphetamine sulfate up to 1.5 times the minimum lethal quantity had no effect in protecting the rabbits from the M.L.D.$_{50}$ or more of alcohol. Indeed, the presence of the

amphetamine increased the toxicity so that the animals tolerated less alcohol (Fig. 6). This suggests a clinical danger which is more apparent than real. In acute alcoholic states in man there is present a quantity of alcohol, which rarely, if ever, approaches a lethal amount, because the hypnotic action of alcohol usually manifests itself long before a lethal quantity can be consumed. In these clinical conditions, therefore, the use of amphetamine sulfate is not likely to be attended with the danger of producing increased toxicity.

In contrast to these findings, alcohol up to 80 per cent of a lethal quantity protected the rabbits from 1.5 times the minimum lethal dose of amphetamine sulfate. This increased protection has been confirmed by Werner,[5] previously cited. Because of the widespread employment of amphetamine sulfate in various disease conditions, the possibility of using alcohol as an antidote in states of acute amphetamine poisoning should be kept in mind.

The possibility that these manifestations are due to disturbances in absorption must be considered. However, if the amphetamine interfered with the absorption of the alcohol, there should be an increased protection against alcohol when large doses of alcohol and small doses of amphetamine are used together, instead of an increased toxicity. Likewise, even if the alcohol interfered with the absorption of amphetamine, this would not explain the occurrence of the increased toxicity to alcohol when large doses of alcohol and small doses of amphetamine are employed together.

SUMMARY

Four hundred twenty-three rabbits were selected as to age (6 to 15 months), weight (1.70 to 3.00 kg.), sex (equal numbers), and given solutions of amphetamine sulfate and/or ethyl alcohol by stomach tube. The standard laboratory diet was withheld for over sixteen hours before and after administration.

The following data were obtained:

(1) The administration of 5 Gm. per kilogram of alcohol to 13 animals produced narcosis (inability after painful stimulus to hop once without losing balance) with an onset of 17.4 minutes and a duration of 408 minutes (average). (2) The administration of 5 Gm. per kilogram of alcohol with 1 M.L.D.$_{50}$ (85 mg.) of amphetamine per kilogram to another 13 animals produced narcosis with an onset of 55.9 minutes and a duration of 307 minutes (average). The addition of amphetamine delayed the onset of the narcosis about 200 per cent and shortened it about 25 per cent.

(3) The M.L.D$_{50}$ of 25 per cent alcohol (38 animals) was 7.25 Gm. per kilogram. (4) The M.L.D.$_{50}$ of 25 per cent amphetamine (68 animals) was 85 mg. per kilogram. (5) The M.L.D.$_{50}$ of mixture A (1 M.L.D.$_{50}$ of alcohol and ½ M.L.D.$_{50}$ of amphetamine per unit volume) (63 animals) was 6.025 Gm. per kilogram of alcohol with 35.32 mg. per kilogram of amphetamine. (6) The M.L.D.$_{50}$ of mixture B (1 M.L.D.$_{50}$ of alcohol and 1 M.L.D.$_{50}$ of amphetamine per unit volume) (55 animals) was 5.78 Gm. per kilogram of alcohol with 67.72 mg. per kilogram of amphetamine. (7) The M.L.D.$_{50}$ of mixture C (½ M.L.D.$_{50}$ of alcohol and 1 M.L.D.$_{50}$ of amphetamine per unit volume) (99 animals) was 125 to 140 mg. per kilogram of amphetamine with 5.329 to 5.968 Gm. per kilogram of alcohol.

CONCLUSIONS

In the rabbit (1) amphetamine sulfate has an antagonistic effect on the narcosis of moderate amounts of ethyl alcohol. (2) It does not have an antagonistic action on the narcosis of lethal doses, and even increases the toxicity of near-lethal quantities. (3) Ethyl alcohol, on the contrary, protects the animal from 1.5 to 2 times the M.L.D.$_{50}$ of amphetamine sulfate.

In man these findings may be correlated with the antagonistic effect of amphetamine sulfate in acute alcoholic states. Inasmuch as such conditions involve only moderate quantities of alcohol, and rarely, if ever, near-lethal amounts, the possibility of increased toxicity from the amphetamine sulfate-ethyl alcohol combination is remote.

Alcohol may be of value as an antidote in states of acute amphetamine poisoning.

Grateful acknowledgment is made to Dr. M. S. Dooley for advice and criticism, to Dr. H. P. Smith for statistical analyses of the results, and to Dr. John Rowlingson for technical assistance.

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TOXIC REACTIONS TO SULFAPYRIDINE*

Acute Hemolytic Anemia, Leucemoid Reaction, and Purpura in Three Separate Cases

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SULFAPYRIDINE, like most other potent chemotherapeutic agents, has concomitant side effects and toxic reactions. A knowledge of these facts cannot be too strongly stressed for the safe administration of the drug. The deleterious effect of sulfapyridine on hemopoiesis of susceptible individuals is among the most dangerous of its toxic actions. Several cases[1-6] of bone marrow depression, as evidenced by leucopenia with agranulocytosis or thrombocytopenia, have been reported from large or even small doses of the drug. This is not surprising, since the benzene ring is present in sulfapyridine and is capable of causing blood

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reactions affecting the formed elements of the blood either singly or in combination, producing acute hemolytic anemia, unusual leucocytosis, or leucemoid blood pictures, leucopenia with malignant neutropenia, or agranulocytosis and thrombocytopenia with hemorrhagic phenomena.

To date, there has been only one report[7] of leucemoid reaction due to inordinate stimulation of the bone marrow by sulfapyridine. It is, therefore, felt worth while to describe three additional cases of serious bone marrow damage caused by this drug, which produced this leucemoid blood picture in addition to severe hemolytic anemia in the first two patients, and thrombocytopenic purpura in the third patient.

CASE REPORTS

CASE 1.—F. C. (No. 105350), a 25-year-old male was admitted on Dec. 25, 1939, and discharged on Jan. 14, 1940. He complained of fever, chills, cough with expectoration of greenish, blood-stained sputum, and pain in the right chest for six days before admission. Physical examination revealed an acutely ill colored male with tachypnea, widened alae nasi, dullness of right upper chest anteriorly, where bronchovesicular breathing and crackling râles were heard. The clinical diagnosis of pneumonia of the right upper lobe was corroborated by x-ray taken the day of admission.

The urine, blood glucose, and nonprotein nitrogen on admission were normal. The Wassermann test was negative.

The patient was given 30 grains of sulfapyridine on admission (Dec. 25, 1939) and 15 grains every four hours until Jan. 1, 1940. The temperature, which was 102° F. on admission, dropped to 99° F. by December 27, and stayed at a normal level until December 31, when it rose to 101° F. for four days. The secondary rise in temperature on December 31, was due to the action of the drug causing a severe hemolytic anemia (Table I) and not to the pneumonic process which showed complete resolution both by physical signs and x-ray on Jan. 1, 1940.

It will be noted that the hemoglobin of 95 per cent on admission fell to 60 per cent after four days of the administration of sulfapyridine, and the red blood cells fell from 4.5 to 3 million. There was a marked leucocytosis (31,000) with the appearance of many premature forms in the circulating blood. By Jan. 1, 1940, the red blood cells had fallen to exceedingly low proportions: the hemoglobin fell to 20 per cent with 1.5 million red blood cells. The white blood count rose to 64,800. The patient now exhibited an icteric tinge of the skin. The icteric index was 10; the van den Bergh was positive and delayed; urobilin excretion was increased in the urine at this time; wet preparation for sickling of erythrocytes was negative. The sulfapyridine was immediately discontinued, and a blood transfusion was given on the same day; this was repeated two days later. A bone marrow puncture revealed a hyperactive erythronormoblastic marrow (Table I). When the temperature fell to normal on January 4, the blood rapidly returned to normal, and by January 8 no abnormal elements were seen in the blood. The patient's clinical condition improved rapidly and he was discharged, fully recovered, on Jan. 14, 1940.

The marked acute hemolytic anemia and the marked response of the granulocytic cells with many immature forms produced a leucemoid blood picture in this case, which lasted only a few days and responded to blood transfusion after discontinuance of sulfapyridine.

CASE 2.—J. T. (No. 106133), a 49-year-old male, was admitted on Jan. 17, 1940, and discharged on Feb. 11, 1940. The patient complained of pain in the left chest, with fever, cough, and expectoration of thick sputum for four days.

Physical examination revealed an acutely ill patient with a temperature of 103° F. There were absolute dullness, bronchophony, bronchial breathing, and crackling râles at the left base of the lung. The clinical diagnosis of left lower lobe pneumonia was corroborated by x-ray on Jan. 17, 1940.

The patient was given 30 grains of sulfapyridine on admission. This was repeated four hours later, and then 15 grains were given every four hours. On January 21 and 22, icterus developed. The blood count (Table II) showed rapidly developing anemia. The sulfapy-

ridine was discontinued on January 22, and blood transfusion was given. This was repeated on January 24 and January 28. As the blood chart shows (Table II), there was a milder leucemoid reaction in this case than in the former one (Table I), the height of the leucocyte rise being only 28,750. Numerous abnormal cells were found in the circulating blood. The hemolytic process also seemed milder than in the first case. Sickling in wet preparations was negative. The bone marrow in this patient (Table II) was similar to the previous case and showed a hyperplastic erythronormoblastic tendency. When the patient was discharged, he had fully recovered from the toxic effects of sulfapyridine on the blood.

CASE 3.—M. K., a 50-year-old female, was admitted on Nov. 23, 1939, and discharged on Jan. 7, 1940. Her illness began on November 18, with pain in the chest, cough, chill, and blood-tinged sputum. A diagnosis of pneumonia was made prior to admission and about 60 grains of sulfapyridine were administered daily. She entered the hospital because of the increase in chest pain and rise in temperature.

Physical examination revealed marked dullness, increased tactile and vocal fremitus, and fine crepitant râles over the right middle and lower lobes. The clinical diagnosis of pneumonia was substantiated by x-ray on November 24, showing clouding in the right side, with suggestion of some interlobar pleural thickening.

The admission temperature was 103° F. Maintenance doses of sulfapyridine (90 grains in all) were administered. The temperature declined to 98° F. on the third day, when bright red blood was expectorated. Two hemorrhagic papillomatous masses were observed on either side of the buccal mucous membrane near Stensen's duct. There were elevated red papillae at the borders of the tongue and hemorrhage in the gingival tissue of the lower jaw. In addition, there was moderate vaginal bleeding. That night a generalized rash was noticed, with hemorrhagic papillomatous masses throughout the buccal mucous membrane. There was also hemorrhage into the left sclera. The sulfapyridine was discontinued, and a blood transfusion was given with 500 c.c. of saline and coagamine (thromboplastin). The following day the patient showed marked pallor. Purpura appeared in scattered patches over the trunk and extremities, as well as a large ecchymotic area over the left thigh. This constituted the height of the toxic reaction. With transfusion, coagamine, and time the patient recovered and was discharged well, except for some chronic pneumonitis and pleural thickening as a residue of her pneumonia.

As a study of the blood shows (Table III), this patient entered the hospital with an anemia (54 per cent hemoglobin, 3,500,000 red blood cells), undoubtedly due to the administration of sulfapyridine. The anemia alone was not considered sufficiently profound to withhold sulfapyridine because of the clinical picture of the patient. The purpuric manifestations which developed on the fourth day were of the secondary type of thrombocytopenic purpura, with low platelet count (25,000), prolonged bleeding time (twenty-five minutes), and delayed clot retraction time. The leucemoid reaction in this patient (35,000 W.B.C. with 12 per cent neutrophilic myelocytes) was less marked than in the first case, but still forms an important part of this patient's toxic reaction to sulfapyridine. The bone marrow study here was not significant, because it was taken after transfusion, and it failed to show depression or hyperactivity (Table III).

DISCUSSION

Although the two drugs sulfanilamide and sulfapyridine are closely related, and their toxic effects are quite similar, this discussion is concerned with the latter drug, and shall be limited to its toxic action on the blood. This is probably the most important toxic effect of sulfapyridine, since it occurs not infrequently, is usually severe, and occasionally is fatal. Some degree of anemia is reported in fully 3 per cent of 3,000 cases that received sulfapyridine.[8] The anemia produced by the drug is of the acute hemolytic type, usually occurs during the first three or four days of therapy with rapid hemolysis of the red blood cells, and a rise in serum bilirubin. There is a rapid decline in hemoglobin and red blood cells to alarmingly low levels of 20 per cent, and even

TABLE 1

CASE 1. PERIPHERAL BLOOD

DATE	HB. %	R.B.C.	W.B.C.	NEU-TRO-MYEL.	META-MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	BASO.	RETIC. %	NORMO-BLASTS 100 W.B.C.	RED BLOOD CELL MORPHOLOGY
12/25	95	4,50	17,0			18	59	20	3					
12/29	60	3,10	31,0	8		38	27	13	14					
1/1	20	1,50	64,8	3	8	35	45	8	1				15	Anisocytosis, poikilocytosis, hypochromia, polychromatophilia
1/2			60,0	1	12	35	45	6			1	16.4	23	Anisocytosis, poikilocytosis, macrocytosis, polychromatophilia
1/3	44	1,65	46,2		15	20	51	9	5					Anisocytosis, poikilocytosis, macrocytosis, polychromatophilia
1/5	60	2,00	7,25		2	11	59	20	8			25.6	7	Anisocytosis, poikilocytosis, macrocytosis, polychromatophilia
1/8	70	3,15	6,00			5	65	23	3	3	1	7.2	Rare	Anisocytosis, poikilocytosis, macrocytosis, polychromatophilia
1/11						1	60	34	3	1	1	3.2	Rare	Anisocytosis, poikilocytosis, macrocytosis, polychromatophilia

CASE 1. BONE MARROW IN THE ANEMIC PHASE

DATE	TOTAL COUNT	MYELO-BLASTS	PRO-MYEL.	NEU-TRO-MYEL.	EOS. MYEL.	META. MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	PLASMA CELL	MEGALO-BLASTS (PER 100 W.B.C.)	ERYTHRO-BLASTS (PER 100 W.B.C.)	NORMO-BLASTS (PER 100 W.B.C.)	REMARKS
1940 1/2	542,000			5	2	24	30	34	3		2		6	79	185	Hyperplastic
In Recovery Phase																
1/11	64,8		2	8		15	17	36	15	2	5		2	12	62	

TABLE II

CASE 2. PERIPHERAL BLOOD

DATE	HB. %	R.B.C.	W.B.C.	NEU-TRO-MYEL.	META-MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	BASO.	RETIC. %	NORMO-BLASTS 100 W.B.C.	RED BLOOD CELL MORPHOLOGY
1/17	72	3,51	11,2			33	58	9						
1/22	32	1,81	25,4	2	11	21	49	8	6	3			7	Anisocytosis, Poikilocytosis
1/23	30	1,11	25,0	1	7	28	53	4	7			4	7	Anisocytosis, Poikilocytosis
1/24	32	1,70	28,7	1	5	14	63	11	4	2		9.4	2	
1/25	43	1,76	17,15			13	74	9	4			13.4		
1/29	58	2,69	11,15				66	24	8		2	8.4		Anisocytosis, Poikilocytosis
1/31	60	2,81	12,12				66	26	8					
2/4	74	3,43	9,55				65	24	9	2		0.6		
2/8						1	30	56	10	2	1	0.5		

CASE 2. BONE MARROW IN THE ANEMIC PHASE

DATE	TOTAL COUNT	MYELO-BLASTS	PRO-MYEL.	NEU-TRO-MYEL.	EOS. MYEL.	META. MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	PLASMA CELL	MEGALO-BLASTS (PER 100 W.B.C.)	ERYTHRO-BLASTS (PER 100 W.B.C.)	NORMO-BLASTS (PER 100 W.B.C.)	REMARKS
1940 1/23			2	11	2	20	35	21	5		2	2	7	32	111	Hyper-plastic
In Recovery Phase																
2/8	66,8	1	3	12	2	22	24	17	12	1	5			2	37	

TABLE III

CASE 3. PERIPHERAL BLOOD

DATE	HB.	R.B.C.	W.B.C.	PLATE-LETS	NEUTRO-MYEL.	META-MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	BASO.	RETIC. %	NORMO-BLASTS 100 W.B.C.	RED BLOOD CELL MORPHOLOGY
1939															
11/27	54	3,50	23,8												
11/27	54	3,37	28,3	25,000	2		22	63	11	1	1				
11/29	44	2,80	33,0	25,000	12	15	25	33	11	2	2		2	1	Slight anisocytosis, poikilocytosis
12/1	43	2,40	19,5		8		26	39	15	6	5				
12/5	63	3,24	11,1	355,000	3		14	42	26	11	3	2			
12/8	63	3,44	9,8	250,000	1		7	62	28	2					
12/11	62	3,09	8,1	325,000	3		10	48	30	8	1				
12/16	62	3,80	9,7				10	54	32	4					
12/20	66	3,60	6,3	250,000			7	52	33	6	2				
1940															
1/2	75	3,30	7,2				3	45	52						

CASE 3. BONE MARROW

DATE	TOTAL COUNT	MYELO-BLASTS	PRO-MYEL.	NEU-TRO-MYEL.	EOS. MYEL.	META-MYEL.	NON-SEG.	SEG-MENT	LYM.	MONO.	EOS.	PLASMA CELL	MEGALO-BLASTS (PER 100 W.B.C.)	ERYTHRO-BLASTS (PER 100 W.B.C.)	NORMO-BLASTS (PER 100 W.B.C.)	REMARKS
1939																
12/2			3	4		23	22	34	10		4		1		29	Occasional megakaryocytes; very numerous platelets

to one million red blood cells with all the findings of an acute hemolytic crisis, with the presence of frequent normoblasts, anisocytosis, poikilocytosis, frequent macrocytosis, hyperchromia, polychromatophilia, basophilic stippling, and reticulocytosis (Fig. 1). The peak of the reticulocytosis occurs at the third or fourth day; in Case 1, to a rise of 25.6 per cent, and in Case 2, to 13.4 per cent. The action of the drug appears to be entirely peripheral, because the few bone marrow studies, including our own, which have been made in these cases, show no depression but rather hyperplasia and increased hematopoietic activity of the marrow. The bone marrow in the first two cases (Tables I, II) showed great cellularity and hyperplasia, especially of the erythropoietic elements, such as an increase in erythroblasts, with normoblasts and occasional megaloblasts (Figs. 2 and 3). This hyperplasia is undoubtedly due to an attempt on the part of

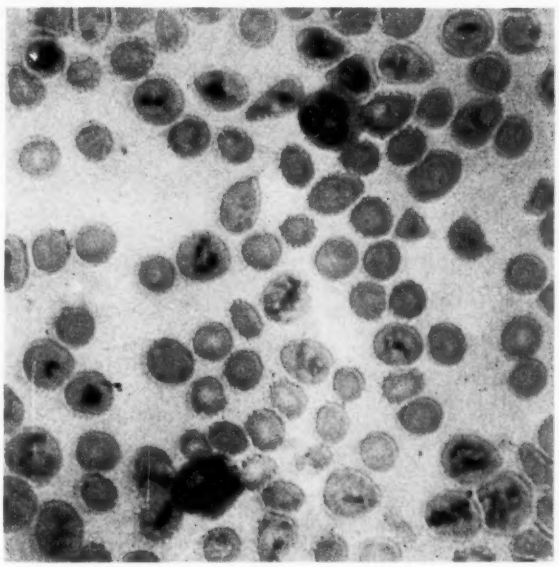

Fig. 1.—Showing reticulocytosis.

the bone marrow to compensate for the rapid intravascular blood destruction in an effort to meet the increasing demands for regeneration in order to combat the anemia. The myeloid elements show a moderate degree of hyperplasia with a shift to the left. Although the hemolysis and anemia are somewhat progressive even after the withdrawal of sulfapyridine, the anemia usually improves with repeated blood transfusions. It is of interest to note that, with clinical improvement showing an increase in hemoglobin and red blood cells, the bone marrow shows a corresponding reduction in erythroid cells.

Since there is no abnormal fragility of the red blood cells in these cases, it may be assumed that they are destroyed directly by some hemolytic process or indirectly through the reticulo-endothelial system. It is known that the effect of sulfapyridine on the blood is often selective, causing hemolytic anemia with moderate leucocytosis. As with sulfanilamide, the most commonly reported damage caused by sulfapyridine is marked reduction of the number of leucocytes with granulocytopenia.[8] The mechanism is probably on a basis of maturation

arrest or hypoplasia of the granulocytic series in the bone marrow, similar to that seen following benzol, aminopyrine, etc. There has been only one report to date of the opposite toxic effects producing a leucemoid picture. Leucemoid reactions have been reported by Heck and Hall[9] and have been observed in overwhelming infections more commonly of coccal than bacillary origin, sepsis, uremia, occasional heavy metal poisons, measles, infectious mononucleosis, hemolytic anemia, polycythemia vera, instances of the lymphomatous diseases, Albers-Schönberg disease, and multiple myeloma. The leucocytosis observed resulting from sulfanilamide or sulfapyridine may be due to actual irritation of the bone marrow rather than to the underlying infection, because it often occurs when

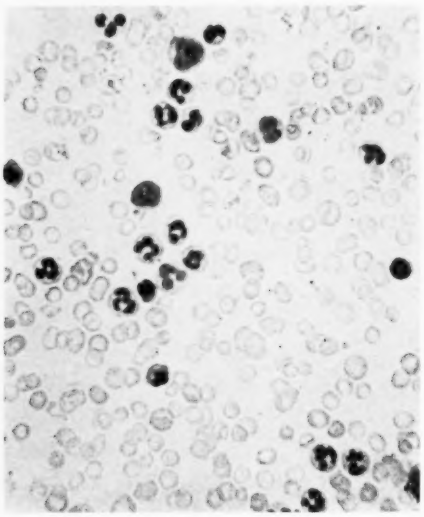

Fig. 2.

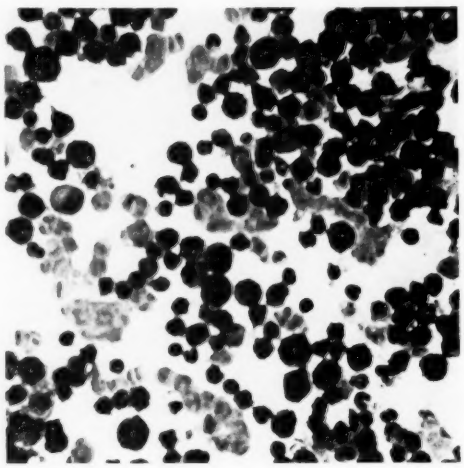

Fig. 3.

Fig. 2.—Peripheral blood smear showing marked leucocytosis with few myelocytes and normoblasts.

Fig. 3.—Hyperplastic erythronormoblastic bone marrow showing numerous erythroblasts, normoblasts, and myeloid elements.

the infection is on the decline. A diagnosis of leucemia is sometimes ventured, but the presence of a potent producing cause, the dramatic clinical improvement of the patient, and the rapid return of the blood to normal should offer no difficulties in excluding leucosis. The case reported by Moody and Knouf[7] is similar to those reported here, showing the rather common acute hemolytic anemia and the very uncommon leucemoid reaction which responds satisfactorily after blood transfusion.

The occurrence of secondary thrombocytopenic purpura with a mild leucemoid reaction, reported in our third case, raises the question of the possible allergic reaction of sulfapyridine on the blood. According to the allergic theory,[10] drugs like sulfanilamide and sulfapyridine produce thrombocytopenic purpura by sequestration or destruction of platelets caused by widespread alteration of the reticulo-endothelial system in persons who have a constitutional predisposition with acquired sensitivity to certain drugs. Although the theory cannot be easily disproved, Hodes and associates[3] offer good evidence that so far as the leucopenia is concerned, the specific hematologic reaction patterns

are not due to acquired sensitivity, because patients who developed leucopenia in their first course of treatment with sulfapyridine failed to show similar reaction during their second course of treatment.

It must be admitted that as yet the exact mechanism in which sulfapyridine affects the blood and blood-forming organs adversely is not known. Nevertheless, we cannot help feeling strongly that a complete blood count should be done to determine the condition of the patient's blood before administration of the drug. Repeated blood counts either daily or at least every other day must be done so that toxic effects on the hemoglobin, red blood cells, leucocytes, and platelets may be readily detected, the drug discontinued in time, and repeated blood transfusion administered to avoid fatal outcome in these unfortunate cases.

CONCLUSIONS

Unusual toxic reactions of the blood to sulfapyridine are reported in three cases with acute hemolytic anemia, and varying grades of leucemoid blood pictures and associated secondary thrombocytopenic purpura. In spite of these serious reactions all the cases recovered in a short time after transfusions. Bone marrow studies showed erythroblastic hyperactivity in the two cases. The theories of the effects of the drug on hemopoiesis are discussed. It is believed that the marked hemolytic anemia may be evidence of a peripheral action; the leucocytosis an irritation of the myeloid element of the bone marrow; and the thrombocytopenic purpura an allergic phenomenon. The importance of frequent blood counts on any patient receiving sulfapyridine cannot be overemphasized.

We wish to thank Professor Linn J. Boyd for the privilege of reporting these cases.

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PLEURAL SHOCK—A REFLEX*

Preliminary Report

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FOR many years the etiology of pleural shock has been debated; men of eminence have suggested that the cause is air embolus, while others of equal importance believe the condition is reflex in origin.

Studies have been made in which blood pressure, pulse rate, and respiration have been carefully checked. These merely serve to confirm the existence of pleural shock, but do not demonstrate its etiology.

It has been the custom here in the surgical laboratory to sacrifice animals by injecting ether into the chest cavity. Almost immediately after the injection the animal has a mild convulsion, rolls on its back, and within two or three minutes is dead, respirations having become rapid and shallow, and finally ceasing; heart action also stopping after what seems like a short period of increase in activity. Pupils become dilated; cyanosis is marked.

It occurred to us that the time elapsed was too short to account for the death on other than a reflex basis, and that the ether could not have caused an anesthetic death in so short a time. We postulated a vagovagal reflex (pleurocardiac) and consulted the literature for support. The reflex was suggested as early as 1865 and has been tentatively confirmed by experimental work in which atropine prevented or diminished signs of pleural shock, as did vagotomy.

We, therefore, sacrificed several rabbits left from other work by injecting ether in the pleural cavity, and taking electrocardiograms throughout the procedure. We have succeeded in killing rabbits with as little as 0.5 c.c. of ether in as little as one minute. Autopsies have revealed no gross evidence of air embolism.

Manometer readings are taken to show that the ether has been injected into the pleural cavity. Pressure increases are relatively small, showing that cardiac embarrassment due to mediastinal shift is not responsible for the deaths.

Electrocardiographic tracings are taken in all three limb leads before insertion of needle into chest. Lead II is then taken after insertion of needle and during injection of ether, and until after clinical death is evident. The records show changes in the T-wave, inversion and flattening being pronounced, and the take-off high.

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We expect to continue this work on rabbits and larger laboratory animals. Tracings will also be made on human beings whose chests are entered for thoracentesis, pneumothorax, and surgery.

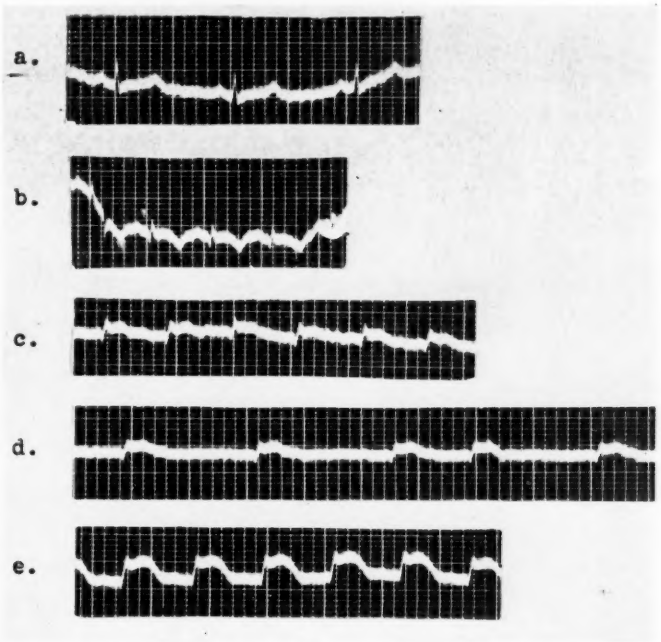

Fig. 1.—*a.* Normal electrocardiogram of a rabbit; P-waves are positive. Initial complex shows low R-wave neither split nor widened. S-T segment is in isoelectric line. T-waves are positive. *b.* Taken 67 seconds after injection, shows tachycardia, short Q-wave. R-wave is lower, T-wave is inverted. *c.* Taken 80 seconds after injection, shows high take-off of S-T segment and T-wave from the descending part of R-wave. *d.* Taken 89 seconds after injection, shows arrhythmia. P-waves are not distinctly visible. Ventricular complex is unaltered. *e.* Taken 131 seconds after injection, shows regular tachycardia with marked high take-off, causing almost a monophasic electrocardiograph.

This report is submitted so that others, dealing mainly with chest pathology and with opportunity to apply this investigation to human beings, may check our early impressions with actual clinical experience.

ENDOCRINE ASPECTS OF HEADACHES

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THE problem of headaches has been investigated from several viewpoints in accordance with the fact that pain may be caused by a variety of pathologic processes affecting the structures of the head, as well as by a variety of systemic conditions, both infectious and metabolic. Headaches also develop in the absence of all organic disease as an expression of disturbances in the emotional sphere or of a psychoneurotic state. However, those either of the migraine, or a less specific type, frequently occur in patients who do not present any, or not enough, organic pathology to account for their complaints; nor can the headaches be blamed on nervous or mental conditions.

The occurrence of headaches in patients who consult the endocrinologist for varied reasons is so common that it seemed justified to investigate the possible causal relationship between endocrinopathies and attendant metabolic disorders on the one hand, and the attacks of headaches on the other.

A series of 50 consecutive cases of severe headaches is presented, most of which were of the characteristic migraine type. All cases, including those clinically not classified as migraine, were noteworthy for their severity, frequency, and refractoriness to ordinary palliative treatment. Organic pathology or systemic disease as the cause of these headaches could be ruled out by thorough clinical examination.

Oliguria was a distinct feature, for the twenty-four-hour urine output was less than 1,000 c.c. in the majority of these cases. The daily urinary volume was above that figure in one-third of the group, with a maximum of 1,780 c.c. in one patient; but all these patients admitted an increased liquid intake. The twenty-four-hour urine specimens showed an average sodium chloride content of only 6.7 Gm. in spite of the fact that a good majority of the patients admitted an increased liking for, and consumption of, salty food.

A salt tolerance test was performed on all patients. It consisted of the administration of 10 Gm. of sodium chloride and 250 c.c. of water in addition to the identical food and liquid intake of the preceding control day. There was a rise of the twenty-four-hour salt excretion to 9.4 Gm. as an average, indicative of a retention of 73 per cent of the test salt for the whole group. Only 6 persons showed complete elimination of the added 10 Gm. of salt. On the other hand, complete retention of the test salt was noted in a similar number of cases, while the chloride excretion after salt intake was actually smaller than on the control day in 8 persons.

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The twenty-four-hour urinary volume averaged 1,040 c.c. for the whole series; it decreased to 947 c.c. on the test day in spite of the fact that the liquid intake was 250 c.c. higher. In other words, the average retention was 343 c.c. It might be mentioned that water was retained in only one of the 6 patients who did not retain salt, whereas the other 5 eliminated increased amounts of urine. Thirty-two patients, i.e., a majority of the group, showed an actual decrease in urine output, and a slight increase, but less than the 250 c.c. of additional liquid intake, was noticeable in the remaining 13.

Estimation of blood chlorides carried out on whole blood was not found enlightening until the study was extended to the examination of chlorides separately in red blood cells and plasma.

The values for chlorides in the whole blood were found within the normal range from 480 to 510 mg. per cent. Higher values up to 530 mg. per cent were obtained only in 10 persons. The sodium chloride content of the red blood cells, however, ranged from 390 to 430 mg. per cent, exceeding the upper limit of our normal figures (380 mg. per cent) in 32 cases, that is, in 64 per cent of the series.

Studies of the sodium content of the blood were carried out only on the last 10 patients of this group. The values found varied between 230 and 264 mg. per cent, whereas control figures obtained with the same methods reveal the normal sodium level at 200 to 220 mg. per cent.

Determination of the basal metabolism showed that the average rate for the group was minus 10; the individual figures varied from minus 27 to plus 13, with the majority of the group well below minus 10. The specific dynamic action of proteins determined two hours after the intake of a small protein meal revealed an average increase of the metabolic rate of only 5 points. No rise at all was noted in almost one-half of the group; a considerable increase of the specific dynamic action of 23 to 28 points was observed in 6 persons. Upon elimination of these few patients who seem to deserve special consideration, the remaining 44 showed an average specific dynamic action as low as 2 points.

The fasting blood sugar ranged from 65 to 108 mg. per cent, with an average of 85 mg. per cent which is close to the lower limit of our normal range. Uric acid values were found to be from 2.2 to 4.8 mg. per cent, with an average of 3.4 mg. per cent which is definitely above normal. The differential count of the white blood cells showed variations of the lymphocyte count from 12 to 55 per cent, yet the average was 36 per cent, definitely connoting a relative lymphocytosis.

DISCUSSION

Our study seems to show the definite tendency in our group of patients to produce a low volume of urine with reduced sodium chloride output. There is marked retention of salt during the performance of the salt tolerance test, accompanied by water retention, which in some of the cases became visible in the form of swellings or seemed to precipitate a migraine spell. Clinically, two facts deserve emphasis: (1) the frequency of demonstrable vasomotor disturbances, such as white dermographism, together with a variety of related clinical manifestations, such as urticaria, Quincke's edema, rhinitis, and "sinus trouble"; (2) the prevalence of endocrine stigmatization. The latter includes,

first of all, the physical appearance of the patients who seem to fall mainly into two groups. One group included patients of both sexes who were tall, lean, and of slightly or definitely eunuchoidal proportions, while most of the patients of the other group showed peripelvic fat deposits such as are thought to be characteristic for pituitary insufficiency. The pattern or changes described as characteristic for a disturbed state of pituitary function (Goldzieher) conforms with the trend demonstrable in this group: low basal metabolism, decreased specific dynamic action of proteins, relative lymphocytosis, increased uric acid, and low normal fasting blood sugar.

That migraine is likely to occur in individuals, earmarked by their physical appearance as being affected with a disturbance of pituitary function, was stressed long ago by Timme, who suggested that the migraine attack might be precipitated by pressure of an enlarged pituitary upon the intracranial tissues. Such an enlargement might be the result of an increased blood and lymph flow to this gland, similar to the directly observable change in volume of the thyroid. Timme deserves credit for having pointed out the frequent coincidence of pituitary stigmatization and habitual headaches in association with angioneurotic manifestations, such as urticaria, hay fever, or asthma. Yet his explanation of increased intracranial pressure as the result of swelling and temporary enlargement of the pituitary is not tenable. X-ray studies show that the sella turcica was, in two of three cases, either enclosed by overlapping clinoid processes, or its aperture was narrowed to such an extent that bulging of the pituitary into the cranial cavity does not seem to be conceivable. An enlarged sella with an aperture wide enough to permit the pituitary to bulge into the cranial cavity was encountered only exceptionally in our series.

The relationship of the pituitary to intracranial pressure, and the causation of headaches, has aroused renewed interest through the findings of Riley, Brickner, and Kurzrok, who demonstrated the appearance of the pituitary gonadotropic hormone in the urine, generally from one to six days before the onset of a migraine spell. The hormone disappeared on the day of the attack, and occasionally appeared without being followed by headache. The excretion of the gonadotropic hormone was practically continuous in two patients with status migrainicus.

The findings of Riley and co-workers gained added significance through the studies of Kraus, who showed that increased intracranial pressure, regardless of its cause, stimulates the pituitary gland and causes hypertrophy of the anterior lobe. This hypertrophy is the anatomic expression of functional overactivity, demonstrated directly by the appearance of the gonadotropic hormone in the urine, and indirectly by the development of follicular cysts of the ovaries (Kraus).

The findings of Kraus permit the reinterpretation of the observations of Riley and associates in the sense that the increased excretion of gonadotropic hormone in migraine cases is the consequence of the increased intracranial pressure that precedes and eventually elicits the migraine attack. That increased intracranial pressure is capable of causing pain needs no argument. As a mat-

ter of fact, pressure or pull on the dura mater, and particularly the dural blood vessels, is considered to be the cause of most headaches (Pickering, Penfield, and Mc Naughton).

The mechanism of these functional headaches can be summarized as follows: Due to intracellular retention of salt, and especially of sodium, there is greater avidity of the tissues for water; the attack ensues if increased permeability of the capillaries, as a result of abnormal irritability of the vasomotors, permits a greater flow of moisture to the tissues. The retention of water and subsequent swelling of the tissues within the rigid cranium causes pain which lasts until the water is released through some process of adaptation in which the pituitary is again concerned in some way. The observation that termination of a migraine attack is often accompanied by polyuria is in accord with this view. As further corroboration, we should add that such headaches are quite common in women in connection with the menstrual period; they set in just preceding the menstruation, at a time when water retention is a physiologic event. These menstrual headaches are the best examples for the prevalence and combination of the two responsible factors: water retention and vasomotor instability. The former is proved by the premenstrual gain and postmenstrual loss in weight, while the increased capillary permeability of the premenstrual period is actually demonstrated by the "blister technique" on the skin of normally menstruating women (Peterson and Miller).

In contradistinction to the mechanism of these headaches, which seems to be clarified, the underlying pathology remains an open question. A constitutional, and often familial, element appears to be one of the factors which includes instability of the vegetative nervous system, particularly in respect to the vasomotors. Environmental factors, such as emotional upsets, nervous strain and fatigue, allergic phenomena, or endocrine disturbances are likely to aggravate the vasomotor neurosis. The influence of hormones upon the function of the capillary system is demonstrable experimentally, and clearly shown by clinical observation on menopausal women or castrates. Their outstanding complaints are referable to vasomotor disturbances, which respond well to estrogens, just as the flushes and allied symptoms occasionally noted in the middle-aged male, are relieved by administration of the male sex hormone.

Vasomotor disturbances alone do not cause headaches unless they are associated with a hydropexic state. The retention of water might be the result of long-continued, adverse nutritional habits, such as the excessive intake of salt, polydipsia, a diet low in proteins and high in carbohydrates with vitamin deficiency, as an added possibility. Barring extreme dietary indiscretions, however, it is unlikely that the normal human body, characterized by its power of adaptation, should be unable to take care of an overabundance of water or of food regardless of how unsuitable its composition. Hence, we must postulate a breakdown in the physiologic regulation of salt and water metabolism, such as that obtained in various endocrinopathies. The prevalence of endocrine stigmatization in patients suffering from headaches fulfills this postulate but does not answer the question as to the nature of the endocrinopathy involved, nor as to the hormonal factors that upset salt and water metabolism.

Deficiency of thyroid function may be one of the causes. It is well known that the myxedematous patient retains water and promptly responds to administration of thyroid extract with increased diuresis. Evidence of hypothyroidism, such as short stature, shortness of extremities, firm and dry integument, low basal metabolism, and elevated blood cholesterol, is obtained in quite a few, but certainly not in the majority, of the patients with endocrine headache.

The significance of the adrenal cortical hormone in respect to salt and water metabolism is shown both in the adrenalectomized animal and the patient with Addison's disease who are unable to retain sodium chloride and water normally. Correspondingly, administration of desoxycorticosterone in excessive quantities causes salt and water retention with increased blood pressure and visible edema. Clinically, however, there is no evidence of abnormal adrenal function in the headache patients, hence it would seem that the cortical hormone has no direct bearing upon our problem.

The opposite can be said in respect to the pituitary. Evidence of disordered function of the piuitary was our most common clinical finding, quite in accordance with Timme's observations. Pituitary hormonal factors of known metabolic activity include both those of the anterior and posterior lobes. The antidiuretic hormone of the posterior lobe is supposed to act chiefly by interference with renal secretion. Important evidence, however, points also to effects directly upon the water content of the peripheral tissues. Experiments on frogs injected with posterior lobe extracts and submersed in water have shown that these animals gain weight by direct absorption and retention of water independently from the function of their kidneys (Brunn). Increased water content of the tissues following injection of posterior lobe extract was actually demonstrated by several investigators (Hines and co-workers, Heller and Smirk). It is significant, moreover, that the antidiuretic effect of the pituitary extract is soon followed by increased diuresis and elimination of chlorides. This justifies the assumption that tissue cells react to the posterior pituitary hormone, first with increased hydration, and subsequently after the effect of the hormone has worn off, with release of water and intracellularly stored sodium chloride.

Anterior lobe extracts have been shown to be diuretic (Teel); it was thought that this effect is referable to the thyrotropic hormone. It has been shown, however, that anterior lobe diuresis is independent of the thyrotropic factor (Mahoney and Sheehan) and is related to metabolic phenomena of a more complex nature (Richter). At any rate, the diuretic effects of the anterior lobe explain the apparent antagonism of the two lobes of the pituitary in respect to diabetes insipidus, for this syndrome develops with impairment of the posterior lobe, but only in the presence of functionally active anterior lobe tissue (v. Hann).

The activity of the anterior lobe enters consideration also in view of its gonadotropic secretion, which is the prerequisite of normal gonad function. The significance of estrogens for water metabolism has been pointed out in recent years by experiments in animals and women (Thorn and associates). The water retention produced by estrogens is in agreement with the fact that women retain water and gain weight at a time when the blood estrogen reaches its peak (At-

kinson and Ivy). Oliguria and refractoriness to diuretic procedures at this time are prevalent (Heilig). This is in striking contrast to the loss of weight and noticeable polyuria with increased elimination of sodium chloride (Thorn and associates) at the time of the postmenstrual low estrogen phase. Swelling of the face, extremities, or other parts of the body, common in the course of the premenstrual retention phase, is likely to subside promptly with the onset of the period (Sweeney).

Among the hydrating factors of hormonal nature, insulin must be mentioned, the injection of which causes water retention and may lead to visible edema. While there is no clinical evidence in our group of patients to inculpate the pancreatic islands and to assume an excess of insulin production, it would seem that the hydrating effects of insulin have considerable bearing upon the dietary management of the patient with headache. A diet rich in carbohydrates, especially in those directly convertible into glucose, is likely to stimulate insulin secretion and thus favor retention of water.

TREATMENT

Arguments "ex juvantibus" are usually dismissed as complacent and uncritical. If therapeutic procedures, however, are instituted for the correction of demonstrable metabolic abnormalities, and accomplishment of this task is associated with favorable clinical results, the therapeutic test stands up as additional evidence and should not be ignored. In the whole group of 50 patients, there was only one, a highly neurotic woman, who showed little improvement. It is significant that this patient was one of the few who showed no water retention and only a moderate (40 per cent) retention of the test salt. Forty patients, that is, 80 per cent, were completely and permanently relieved by treatment, while 9 showed considerable improvement both as to frequency and severity of their headaches.

This so successful treatment consisted of three elements: diet, medication, and endocrine therapy. The principles of the diet are largely the same as those outlined by Foldes; restriction of salt and liquid intake, high protein content with emphasis on purine-rich meats (liver, sweetbread, sardines), and considerable reduction in the consumption of carbohydrates. Fats are limited in the overweight, but are liberally given in the underweight patient.

The medication is based on two principles: mobilization and increased elimination of sodium, and correction of the imbalance of the vegetative nervous system. Ammonium chloride or potassium acetate given intermittently accomplishes the former and should be applied in the first two or three weeks of the treatment. Atropine combined with phenobarbital or preparations like belladenal are apt to accomplish the second part of our task. In patients with increased blood pressure, the use of bellergal seems to be preferable.

Endocrine medication consists of thyroid and pituitary extracts. It is fallacious to gauge the dosage of thyroid by the basal metabolic rate of the patient. The actual needs are best determined empirically, starting with 0.5 grain daily and gradually increasing the dosage to not more than 2, or at the utmost 3, grains daily. The patient's pulse rate is the best guide for thyroid

medication; slowing down of the pulse rate calls for increased, and undue acceleration for decreased, thyroid dosage. Injection of posterior pituitary extract is of great value, for increased diuresis and salt elimination follow after the initial antidiuretic effect has worn off. The injections should be properly spaced and given not more often than twice a week. The initial dose is 3 minims, with increments of one minim each time until mild abdominal cramps indicate that tolerance has been exceeded. These injections are given preferably in combination with a fresh and potent anterior lobe extract, especially in the presence of menstrual disturbances or other evidence of pituitary deficiency.

This treatment should be kept up for a period of about three months. Subsequently, both injections and medication are discontinued without the danger of recurrence, provided the spirit of the dietary management is kept alive. In other words, these patients must limit permanently the intake of salt and water and adhere to a high protein, low carbohydrate diet. Rigid dieting is not necessary, but overindulgence either in carbohydrates or in liquid consumption was responsible for relapses in several of our patients. Such recurrences promptly yielded to renewed enforcement of the original diet.

SUMMARY

A series of 50 cases of headaches, observed in endocrine patients, is presented, and evidence is submitted to show a general trend of sodium chloride and water retention. Stigmas pointing to an endocrine background were prevalent; they include: lower basal metabolism, decreased specific dynamic action of proteins, relative lymphocytosis, higher values for uric acid, sodium and chlorides in the blood, lower blood sugar, and abnormalities in the configuration of the sella turcica.

Endocrine headaches, including migraine headaches, are explained as the result of increased intracranial pressure. They develop when increased permeability of the capillaries permits increased flow of water to, and subsequent retention in, tissues which have stored abnormal quantities of sodium salts.

The retentional type of endocrine headache can be recognized by the decreased output of sodium chloride and water on performing the salt tolerance test.

The mechanism by means of which endocrine factors cause increased hydration of tissues is discussed.

The successful treatment of endocrine headaches consists of a high protein diet with restriction of salt, liquid, and carbohydrate intake, of medication with ammonium or potassium salts and belladonna preparations, and of endocrine substitution therapy with thyroid and pituitary extracts.

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THE INFLUENCE OF TIME AND VOLUME FACTORS ON VENOUS PRESSURE RESPONSES TO PHYSIOLOGIC SALINE INJECTED INTRAVENOUSLY*

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COHNHEIM and Lichtheim[1] demonstrated that a 0.06 per cent saline solution would elevate venous pressures without markedly affecting the arterial blood pressure. This finding was corroborated by subsequent investigators,[2-6] who discovered that the systolic output and the size of the heart in diastole were dependent on the elevation of the venous pressure. Caughey[8] suggested that venous pressure responses to intravenous saline be adopted as a test of cardiac function in human subjects, for which purpose he injected 1,500 c.c. of normal saline in thirty minutes. Gilligan and co-workers[7] observed venous pressure responses to intravenous normal saline or to a 5 per cent glucose salt solution in hospital patients without heart disease. They concluded that, although venous pressure variations are dependent on the volume of fluid injected, the increment of venous pressure increase did not necessarily follow the degree of blood dilution.

Studies indicate that the time interval, and the volume of fluid injected are the factors which may modify venous pressure responses. Caughey controlled the time interval without correcting for the volume of fluid injected. Gilligan

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studied blood dilution with variations in the time interval. No experiments are reported in which both the time and volume factors were controlled and studied. In this study either the time interval or the volume factor was constant, while the other variable was changed in order to determine the limits within which these factors influence venous pressure.

METHOD

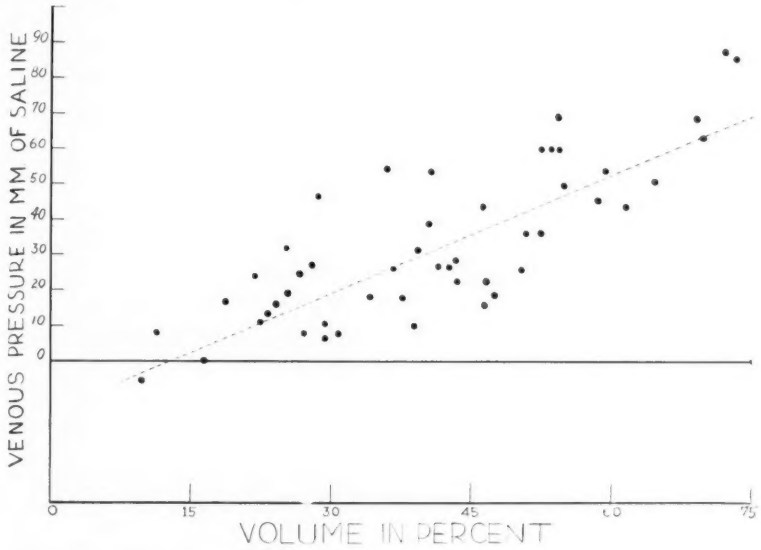
Normal healthy adult dogs were anesthetized with intraperitoneal injections of 30 mg. per kilogram of sodium pentobarbital.* One and one-half inch No. 18 needles were used both for injection and measurement of venous pressure. Normal saline solution heated to body temperature was injected at a continuous rate into the vein of the foreleg by means of hydrostatic pressure from a graduated flask. Direct venous pressure measurements were made from the vein of the hindleg with a continuous drip saline manometer devised for the experiment. In a manometer of this type the respiratory movements may be observed continuously, while the constant flow of 15 to 20 drops of saline solution per minute prevented coagulation of the blood within the needle. At a rate of 15 drops per minute, 33 mm. of saline are necessary to overcome the resistance of the rubber tube and of the needle distal to the manometer. With the bottom of the manometer at the approximate center of the heart, the 33 mm. factor was subtracted from the observed readings in order to obtain actual venous pressure values. Respiratory fluctuations, and the velocity with which the added saline in the manometer dropped to the level at which pressures were in equilibrium, were the means used to check for obstruction within the measuring needle. The blood volume was calculated as one-eleventh of the body weight, and the normal saline injected is expressed as a percentage of this estimated volume. The change in venous pressure during the course of the experiment is expressed in millimeters of saline. In the shorter experiments venous pressure and volume inflow readings were observed every two or three minutes, whereas in the longer experiments these observations were usually checked every five minutes. A total of 83 experiments on 25 dogs were carried out. Ten control experiments in which venous pressures were measured for fifteen-minute intervals showed the normal variations under surgical anesthesia to be less than ± 10 mm. of saline. Fifty experiments were performed with the time interval fixed at fifteen minutes, while the inflow was changed. Another 23 experiments were carried out with the fluid inflow fixed at approximately 50 per cent of the calculated blood volume, while the time was varied.

RESULTS

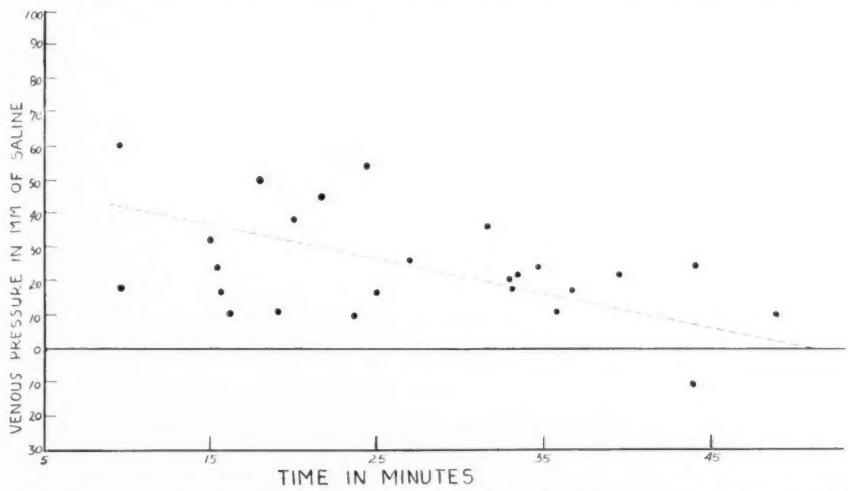
The results have been expressed in graphic form for sake of brevity, and to illustrate the variability of response in normal dogs under surgical anesthesia. Each point in Graphs 1 and 2 represents the total fluid inflow, and the change of venous pressure in millimeters of saline during the course of one experiment. The broken lines representing the most probable response have been drawn so as to divide the points equally.

*Donated through the courtesy of the Abbott Laboratories.

Graph 1 illustrates that, with the time interval fixed at fifteen minutes, greater volumes of normal saline produce greater increments in venous pressure. Volumes greater than 10 per cent of the blood volume have to be injected in this time interval before any increase in venous pressure may be expected. Injections equal to 75 per cent of the blood volume in fifteen minutes produced the maximal response of venous pressure rise to 89 mm. of saline.



Graph 1.—Fluid injected expressed in percentage of estimated blood volume.

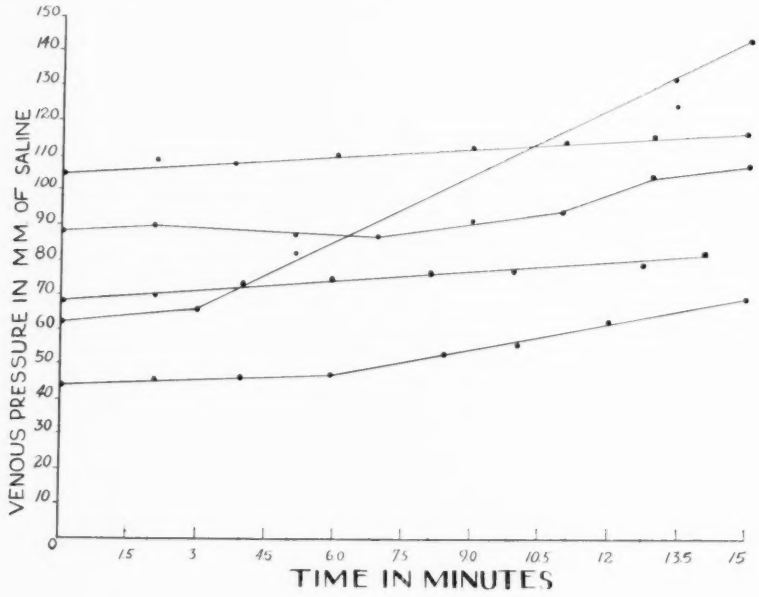


Graph 2.—Saline injected equals 50 per cent estimated blood volume. The volume is constant and the time interval is altered.

Graph 2 shows that with the amount of normal saline injected maintained at approximately 50 per cent of the blood volume; as the time interval of injection is prolonged, the increase in venous pressure becomes progressively less, so that beyond forty-five minutes an animal may show no increase in venous pressure.

Graph 3 shows five individual experiments (numbering from top to bottom) representative of the whole group.

The majority of the responses were either gradual increases or ones that showed abrupt breaks as do curves 4 and 5. Straight lines usually occur when the volume of fluid injected is less than 35 per cent of the calculated blood volume. Changes in the slope of the line develop when the volume injected is 40 per cent or more of the calculated blood volume. Increases in venous pressure appear to be directly proportioned to the volume of saline solution injected. Curves like 2 follow either alterations in velocity of fluid injection or an increase in resistance to the flow of saline from the manometer, due to the poor position of the needle within the vein.



Graph 3.—Individual responses.

DISCUSSION

The relationship between volume of fluid injected and the elevation in venous pressure appears more evident if the time interval is less than fifteen minutes. When the time interval is forty-five minutes or more, diffusion and vasodilatation can compensate fully, so that injections of normal saline equal to 50 per cent of the calculated blood volume may show no elevation in venous pressure. Injections of physiological saline at constant velocities will produce venous pressure increases closely approximating a straight line; shifting the velocity inflow in either direction will cause a corresponding change in the slope of the venous pressure curve. Even though different animals vary as much as 50 mm. of saline for the same volume of fluid injected, on repeated trials in any one dog the results agree closely. None of the animals developed cardiac decompensation following these injections or showed any deleterious effects on the following day.

Moderate pressure, produced by placing one's hand on the animal's chest, caused an increase of venous pressure from 4 to 15 mm. of saline. During the experiment the heart rate usually accelerated and the apex impulse became more forceful. If the animals shivered or stretched their extremities, venous pressures would increase from 10 to 30 mm. of saline. Respiratory movements showed that inspiration produced depression in the venous pressure measuring 2 to 5 mm. of saline, which returned to the original level during expiration. The majority of initial venous pressure readings of saline were between 50 and 70 mm., the lowest value was 29 mm., and the highest was 105 mm.

CONCLUSIONS

1. Both the volume of normal saline injected and the time interval are capable of altering the response of venous pressure to intravenous fluids.

2. With a fixed time interval of fifteen minutes, volumes less than 10 per cent of the calculated blood volume may cause no change in venous pressure.

3. Volumes of saline equal to 50 per cent of the calculated blood volume will show a slight or no increase in venous pressure if the time interval exceeds forty-five minutes.

4. Intravenous saline solution equal to 75 per cent of the calculated blood volume injected in fifteen minutes is tolerated without harmful effects.

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CARDIOSPASM*

Observations on the Use of Prostigmine: A Clinical and Experimental Report

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CLINICAL and experimental observations on the use of prostigmine as an aid in the treatment of cardiospasm and mega-esophagus are reported. The accepted therapy for cardiospasm in the past has been (a) gastric lavage; (b) dilatation of the cardia; (c) the use of antispasmodics. Psychotherapy, diathermy,[1] and vitamin B_1[2] have been reported to be of value. These measures have been effective to a degree in relieving the patient of discomfort and in improving his general nutrition. The esophagus has in most instances remained the same size, and patients have had recurrent symptoms but with a minimum of discomfort despite the large esophagus. Much evidence has accumulated recently to show that in a number of instances cardiospasm is related to damage to vagus fibers or to damage and loss of ganglion cells of the myenteric plexus of the esophagus at the cardia.[3]

The theory of Hurst and associates[4] proposed that following destruction of the parasympathetic myenteric plexus, the sympathetic nerves act unopposed and keep the cardia closed, so that the normal opening reflex of the cardia cannot take place at swallowing[5, 6] and dysphagia occurs, with gradual dilatation of the esophagus. This theory is supported indirectly by observations of Klee[7] on the cat: after section of the splanchnic nerves the cardia lost its power to open reflexly. This is comparable to the tonic "locking" of certain muscles in mollusks after sectioning of their motor nerve. The term "achalasia" is certainly better than "cardiospasm," although it may not cover the entire field of clinical experiences. It is difficult to conceive, f.i. that degenerative changes in the myenteric plexus of the esophagus occur in cases of functional obstruction of the cardia in hysteria; or in reflex disturbances from the abdominal viscera which sometimes improve after operation (cholecystitis, appendicitis, etc.). Recently, intractable cardiospasm has been relieved successfully by bilateral cervicothoracic sympathetic ganglionectomy.[8] This may be considered as proof of sympathetic achalasia, although a longer period of time is necessary for judging the permanency of the beneficial effects of the operation.

From the foregoing, drug therapy for achalasia and mega-esophagus can be elaborated on a logical basis, assuming that we are dealing with a true

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achalasia, that is, a condition in which degenerative changes have taken place in the myenteric plexus of the lower esophagus, through which the parasympathetic nerves effect normal peristalsis. Drugs that affect the parasympathetic system may be used to produce increased tonus and peristalsis of the esophagus, and thus push the food into the stomach. At first, we assumed that some parts of the plexus, upon which these drugs would act, were still functioning. Later we found that certain parasympatheticotropic drugs act not only on parasympathetic endings, but also on the smooth musculature directly (v.i.). Eserine and prostigmine were employed, the latter being the drug of choice because it lacks the disagreeable by-effects of the former.

We first made observations on two patients with very pronounced dilatation of the esophagus of eleven and twelve years' duration. The histories are briefly summarized below. At the beginning of the study we made certain that there was no organic stricture or stenosis of the esophagus. This is a precaution which we feel is necessary to emphasize.

M. F., female, aged 21 years, was admitted to the Michael Reese Hospital on May 8, 1938, because of right lower lobe pneumonia. During convalescence the patient reported that she had a "stricture" of the esophagus and difficulty in swallowing food. The history revealed that the symptom began rather suddenly at the age of 12 and was associated with a severe emotional conflict with her parents. At this time she was admitted to the Sarah Morris Hospital and treated for esophageal "stricture" by dilatation. After a short stay in the hospital her condition improved and she was discharged. In 1929 she was readmitted to Michael Reese Hospital because of a cough and loss of weight. A dilated esophagus was noted. Fluoroscopy and roentgen examination (July 21, 1929) showed mega-esophagus and cardiospasm. Following repeated dilatation of the cardia her condition again improved. Studies during the patient's convalescence on her last admission to the hospital on May 19, 1938, revealed a marked dilatation of the intrathoracic and apparently diaphragmatic portion of the esophagus, with a constriction just above the stomach. On May 27, 1938, an attempt to pass a dilator bougie through the cardio-esophageal hiatus was unsuccessful. The patient was given physostigmine per os 1/150 grain daily, with daily gastric lavage followed by dilatation with a Mosher dilator for one week. This made her feel better and she had less difficulty in getting the food to "go down." She was discharged from the hospital on June 16, 1938, and observation was continued in the clinic. At this time the following routine procedure was pursued: Dilatation was discontinued. Prostigmine* was given by mouth, 15 mg. three times daily, and the patient was advised to return to the clinic in four weeks. At the end of this period she was reported to be improved. Swallowing of solids was better, and she did not need to drink fluids to force food down. Nevertheless, the patient requested dilatation since she felt that this procedure, in addition to the prostigmine, had helped her. Dilatations by the Mosher dilator, at approximately two-week intervals, and then at monthly intervals, were continued. Roentgenograms and fluoroscopic observations of the esophagus were made over the period, and it appeared that the esophagus was smaller.

We have observed this patient for over two years. During periods, when prostigmine was withheld, the patient would return with more complaints and ask that the drug be continued. This was true even though she had had periodic dilatation of the cardia. The patient had been dilated previously at other institutions, but she had not experienced the same degree of improvement as from prostigmine and dilatation. She had also undergone an extensive course

*Supplied by Hoffmann-LaRoche Co.

of psychotherapeutic treatment at another institution with no apparent improvement. The patient admits that her symptoms are aggravated during severe emotional strain.

As a final check, we made fluoroscopic observations of the effect of the injection of prostigmine on the esophagus of this patient. Under fluoroscopic observation a barium meal was administered and 1 mg. of prostigmine was injected intramuscularly. Fifteen minutes later increased peristalsis and tonus (which reached its peak in twenty minutes) were observed. The increased contractions of the esophagus were particularly marked above the cardia, and the barium meal was emptied rapidly into the stomach. Intramuscular injections of 0.5 mg. of adrenaline hydrochloride had no visible effect on the esophagus.

R. N., female, aged 40 years, was admitted to the Michael Reese Hospital on August 6, 1937, after five days of repeated emesis. The past history revealed that she had a cholecystectomy in 1928, and subsequent to this had developed a large esophagus for which she had repeated dilatation with moderate relief. In 1933 the patient had a thyroidectomy. She was an emotional person, with quite a complicated social background. X-ray revealed an unusually large esophagus. The patient was placed on prostigmine, per os, 15 mg. three times daily, and later twice daily, and has been receiving same intermittently for approximately two years. At first she seemed to be distinctly improved, but as she became adjusted to the huge dilatation, it was most difficult to determine what symptomatic improvement we were obtaining. Both dilatation by Mosher dilator and prostigmine seemed to improve her condition, and like the other patient, she expressed the opinion that the prostigmine made her feel better. On the other hand, she had so many emotional conflicts that it was hard to evaluate the improvement.

The effect of prostigmine (and other drugs) on the esophagus was tested under fluoroscopic observation. At 9 A.M. 1/150 grain of atropine sulfate was injected intramuscularly. The patient's blood pressure was systolic 110 and diastolic 84 at 10 A.M. At 10:15 A.M. barium milk mixture was given, but the patient managed with difficulty to drink about ¾ cup. The esophagus was seen fairly filled, with shallow peristaltic waves traveling downward. At 10:30 A.M. 1 c.c. of 1:2,000 ergotamine tartrate was administered intramuscularly. One minute later much deeper and much more frequent waves were observed moving down the esophagus than before. At 10:50 A.M. the blood pressure was systolic 100 and diastolic 70. More barium mixture was given, and shallow peristaltic waves were observed. One-half milligram of prostigmine was injected intramuscularly. The depth of the peristaltic waves increased within a few minutes, at times giving the impression of contraction rings traveling down the esophagus, and a greater frequency of peristalsis and increase of tone of the organ was observed. At 11 A.M. the blood pressure was systolic 118 and diastolic 85. One milligram of adrenalin hydrochloride was injected intramuscularly. Peristalsis, which after the injection of prostigmine had been present continuously, seemed to be somewhat increased. No untoward effect of the drugs given above was noted. At 11:10 A.M. the blood pressure was systolic 114 and diastolic 75.

We realize the difficulties in evaluating therapeutic results, particularly in this condition. It is well recognized that mere dilatation by any type of dilator will improve the well-being of a patient with achalasia, and will diminish the quantity of esophageal retention. It was our thought, that prostigmine, by acting on the parasympathetic plexus, would increase peristalsis and tone of the esophagus. Our observations definitely show that it does increase peristalsis, particularly in the lower portion of the esophagus. In that sense a propelling action of prostigmine is accomplished. Our patients stated that they were better with prostigmine and dilatation. It must be recognized that the achalasia and esophageal dilatation in our two patients were of long duration and of extreme

character. We suggest the use of eserine or prostigmine in early cases. Likewise, it is suggested that hypodermic medication rather than oral administration be tried in cases of chronic dilatation.

ANIMAL EXPERIMENTS

In order to check the effects of prostigmine on the esophagus, experiments were performed on dogs. The animals were anesthetized with nembutal, and three balloons were inserted into the esophagus at different levels: just above the cardia, into the midportion, and just below the glottis. The balloons were connected to water manometers and were inflated with about 5 cm. of water pressure. Blood pressure was recorded in the usual way with a mercury manometer connected to the carotid artery. In Experiment 1 (Fig. 1) respiration was not interfered with, and the curve showed its rhythmic effects on

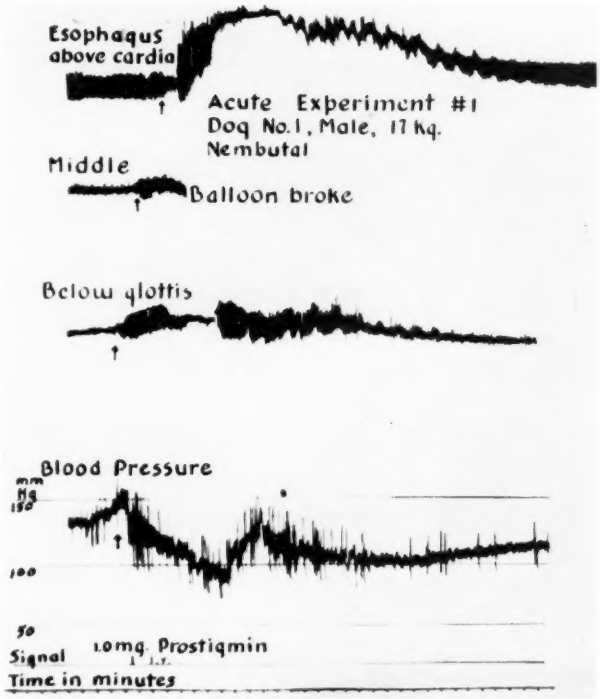

Fig. 1.

esophageal motility. One milligram of prostigmine injected intramuscularly produced a considerable increase of tone and peristalsis in the lower portion of the esophagus, lasting for about twenty minutes, while the upper portion of the esophagus was little affected. The animal in Experiment 2 (Fig. 2) was given artificial respiration, its chest was opened widely, and both phrenic nerves were cut in order to eliminate the effects of respiration on the esophagus. Injection of 1 mg. of prostigmine intravenously produced considerable rise of tone and peristaltic activity in the lowest portion of the organ, much less in the middle and very little in the upper portion. The effects lasted approximately fifteen minutes. In Experiment 3 (Fig. 3) the same operative procedure was followed

as in Experiment 2. Three-tenths milligram of prostigmine intravenously was followed by a rise of tone and motility of the lowest portion of the esophagus, lasting for about twenty-five minutes. The middle portion showed little, and the upper portion showed no effects of the drug. One hour later 1 gamma of acetylcholine hydrochloride was administered intravenously; it had little or no effect on the esophagus while a distinct transient drop of blood pressure occurred. Following this, 4 mg. of atropine sulfate were injected intramuscularly. Twenty minutes later 1 gamma of acetylcholine was injected again, and the negative response of blood pressure indicated that atropine had taken effect.

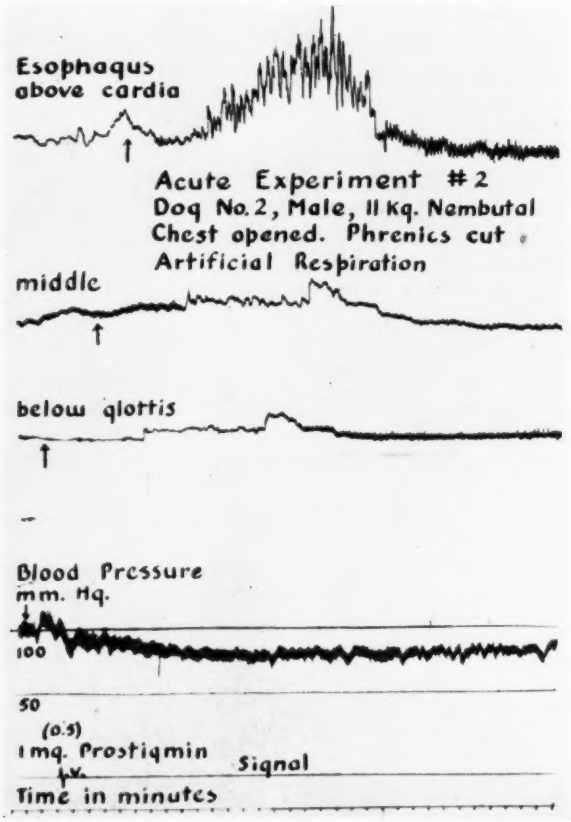

Fig. 2.

Following this, 0.3 mg. of prostigmine was injected intravenously; this dosage was repeated twenty minutes later. Each time a marked rise of tone and contractions of the lowest portion of the esophagus occurred, but the middle and upper portions did not respond. Fifteen minutes after the second injection of prostigmine, 1 gamma of acetylcholine was given intravenously, with no effect on blood pressure. This showed that the antagonistic effect of the atropine still prevailed.

These experiments demonstrated two facts to us: First, that prostigmine raises tonus and rate of peristalsis of the esophagus and acts mainly on its lower third. Second, that prostigmine acts on this organ not only through stimulation of its parasympathetic innervation, but also through a direct effect on its

musculature. We draw this latter conclusion from the fact that prostigmine still affected the organ after the parasympathetics were paralyzed by atropine, as proved by the ineffectiveness of acetylcholine administered repeatedly after atropine. It seems, therefore, that prostigmine is a useful drug in true achalasia in which more or less of the extrinsic and intrinsic parasympathetic innervation of the lower esophagus has been destroyed by pathologic processes. The observations on the dog agree with those obtained on the two patients reported in this paper. The effect of ergotamine on the second patient is of interest, because this drug is known to decrease and, in a sufficient dose, to abolish and reverse the typical effects of sympathetic stimulation. Since in achalasia there is a predominance of sympathetic innervation of the lower esophagus and cardia, it would seem logical to treat such patients with a combination of eserine or prostigmine and ergotamine. This is under experimental investigation at present.

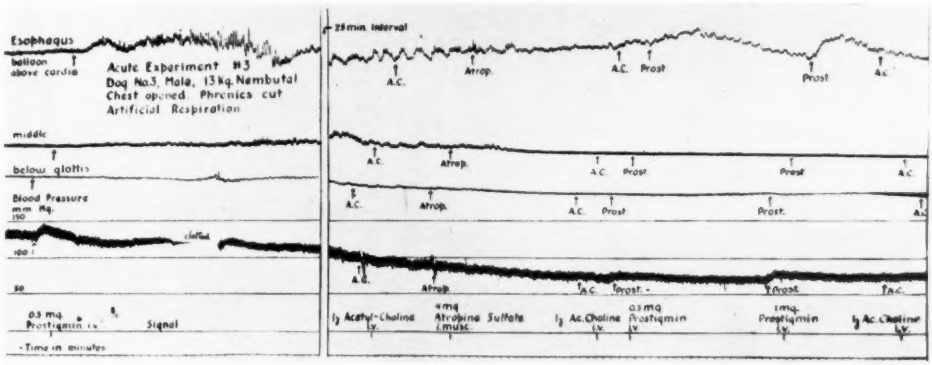

Fig. 3.

SUMMARY

Two patients suffering from achalasia and dilatation of the esophagus were treated by oral administration of prostigmine. Both of them seemed to be benefited by this therapy. Fluoroscopic observations revealed that tonus and peristaltic rate of the esophagus were markedly increased following intramuscular injection of prostigmine. Injection of ergotamine into one patient likewise revealed increased tone and peristaltic rate of the esophagus. Since achalasia of the cardia has been assumed to be due to diminished or absent function of parasympathetic innervation and prevailing sympathetic innervation, prostigmine and ergotamine seem to offer a logical and promising method of treatment of this condition.

Experiments on anesthetized dogs confirmed the observations made on the patients and further reveal that prostigmine acts not only through stimulation of the parasympathetic innervation of the esophagus, but also through a direct effect on its musculature.

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CHRONIC, ATROPHIC TYPE OF BRUCELLOSAL ARTHRITIS

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IT IS well established that different types of systemic infection may cause polyarthritis which, in its clinical appearance and symptomatology, is essentially indistinguishable from true atrophic arthritis. That is essentially true when such arthritis appears in women, in whom atrophic arthritis is most common.

Chronic brucellosis is in course of evolution. At least in the Southwest physicians are becoming increasingly "brucellosis conscious," and rightly so. In keeping with this general trend, and as chronic arthritis has a varied etiology, it is now part of good diagnostic routine to do agglutination and opsonocytophagocytic tests as well as a skin test of each patient for the presence of brucellosis. Reports as to the number of cases of chronic arthritis or chronic fibrositis having positive serologic and skin tests for brucellosis are the subject of a paper in process of preparation. Herein I set forth a syndrome occasionally found in which the pathology is from a clinical standpoint that of atrophic arthritis. Yet, the etiology is that of chronic brucellosis. The two cases presented are representative instances. They occurred in females. Both had typical fusiform spindle-shaped swellings of the interphalangeal joints of the fingers of both hands, as well as involvement of joint tissues in other parts of the body. Joint involvement, from the beginning, was essentially persistent and was not subject to regression. Its development was insidious and progressive. Presence of cold, clammy skin of both hands was noted. Past removal of apparent foci of infection had in general caused no regression of the arthritis. Physical therapy and other measures failed to relieve or to interrupt the disease course. A familial history of arthritis was absent. In one case knee and shoulder joints were definitely involved by a type of fibrous tissue swelling that is often seen in atrophic arthritis. An outstanding x-ray finding was that the bones of the involved joints did not reveal the narrowing of joint space and the decreased density of the ends of the bones forming the joints, which is seen

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in cases of typical atrophic arthritis after the process has been present for some period of time, as was the case in these individuals. Laboratory tests revealed a lack of marked degree of secondary anemia that is often present in atrophic arthritis. Streptococcal antibodies were absent. Gastric analysis was normal, The gonococcal complement fixation test was negative. An active red blood cell sedimentation test was present. The Schilling differential count showed a mild increased shift to the left. An important clinical laboratory finding was a positive agglutination test for undulant fever.

The opsonocytophagic test also revealed a strongly positive test, being in one case as high as 100 per cent mild, moderate, and marked phagocytosis. In both cases skin test was strongly positive at the end of forty-eight hours and for a few days thereafter.

Fig. 1.—*A*, Case 1. M. R. B. X-ray of hands. Chronic arthritis, atrophic type, four years' duration. Note lack of joint space narrowing and demineralization. *B*, Same case as shown in Fig. 1. X-ray of right elbow. Note lack of bony pathology.

CASE 1.—Mrs. M. R. B. was first seen in the summer of 1935, when typical spindle-shaped fusiform swellings involving the interphalangeal joints of nearly all her fingers were present. Her physician husband was informed that it appeared to be a case of atrophic arthritis. She was carefully investigated in due time for focal infection, but none was found to be present. Streptococcal antibodies were not present. Agglutination tests for undulant fever were strongly positive. The opsonic index test was positive. The skin test was strongly positive.

X-ray examination of the involved joints of the fingers simply revealed the normal bones that form the involved joints. Diffuse soft tissue swelling could be seen by x-ray shadow. X-ray examination made of her hands in January, 1939, four years after the onset of her trouble, revealed normal joint spaces and bones forming the involved joints, except for very slight lipping in the terminal joints of both fifth fingers. Narrowing of joint space and demineralization, such as one sees in atrophic arthritis, particularly atrophic arthritis present over such a long period of time, were absent.

X-ray examintaion of the right elbow joint was made in September, 1937, because of joint symptoms, but it failed to reveal any bony pathology.

The patient's past history was essentially negative, except for influenza and a miscarriage in 1927. An appendectomy and curettement were done in 1927, as was the removal of one cystic ovary. In the latter part of 1927 chills at intervals appeared, accompanied by wringing sweat and a feeling of stiffness all over her body, the stiffness involving most

markedly both upper extremities, so that for a period of time they could be used only very little; other joints of her body were involved. A tonsillectomy was performed in 1927. Very slowly and gradually she became better of her rheumatism, but a marked nervous condition developed. Treatment for her nervous condition resulted in gradual clearance of her rheumatism. Prior to the onset of the typical symptoms of arthritis in her hands, she felt ill for a long period of time and lost weight. The patient is of the age during which hypertrophic arthritis develops.

As a result of brucella bacterin therapy administered by her physician husband over a period of a year she improved in every way, clinically and from the standpoint of x-ray examination and laboratory findings.

The absence of findings, laboratory, x-ray, or clinical, that would explain her atrophic arthritis, plus positive skin, agglutination, and opsonic index tests for brucellosis, as well as her admirable response to treatment for chronic brucellosis, with no definite relapse after treatment had been completed, justifies labeling this case as one of brucellosal arthritis, chronic, atrophic type. The case illustrates the chronicity of illness that may be present in individuals plus the essential relapses and improvements that occur. This may happen in atrophic arthritis.

Fig. 2.—Case 2. Mrs. R. S. X-ray of hands. Chronic arthritis, atrophic type. Note lack of demineralization and articular cartilagenous involvement.

CASE 2.—Mrs. R. S. was first seen in January 14, 1937. Her history dated back two years, beginning with pain and discomfort in the left shoulder joint. These symptoms appeared in spells at irregular intervals over a period of some few months. Moderate response at first to salicylates occurred. Thereafter the arthritis appeared insidiously, and became worse progressively. Many joints became involved, especially the hands, knees, and feet, as well as the shoulders and elbows. She had some teeth extracted and her tonsils removed. She had taken treatment at bathing resorts. Scarlet fever and whooping cough occurred in childhood. She has had two pregnancies, one of which was aborted with no ill effects. The abortion took place after the onset of her arthritis. The family history is negative.

Physical examination was negative, save for the presence of marked motion limitation of both shoulder joints and right elbow. Cold, clammy perspiration was present in both hands. Wrist and interphalangeal finger joints were indurated. The involved finger joints revealed typical spindle-shaped fusiform swellings seen in atrophic arthritis. Knuckle joints were also enlarged. A low-grade inflammatory infiltration of the fibrous tissues of both knees, with moderate restriction of flexion, was present. Lateral pressure over the phalangeal joints caused pain. Adherent tubes were questionably present on pelvic examination.

X-ray examination revealed a lack of joint space narrowing and an absence of demineralization of the bones forming the joints. A faint shadow of the soft tissue swelling was noted.

Laboratory findings revealed absent streptococcal antibodies and negative gonococcal complement fixation test. Gastric analysis, blood count, urinalysis, and stool examination were negative. The red blood cell sedimentation test showed a moderately active curve. Agglutination test for undulant fever showed strongly positive results in dilutions of 1:50. Opsonic index test for undulant fever revealed mild, moderate, and marked degree of opsonocytophagocytosis to 100 per cent. The skin test for undulant fever was strongly positive 4+ at the end of forty-eight and ninety-six hours.

The patient responded well to brucellosis treatment and at the end of two years is practically cured.

Diagnosis: Brucellosal arthritis, chronic, atrophic type.

Fig. 3.

SUMMARY

1. The two patients presented had clinical objective and subjective findings that would stamp them as cases of atrophic arthritis.

2. X-ray findings, even though the condition had been present in each case for a long period of time when first seen, and for at least two years thereafter in one case and four years thereafter in the second, continued to reveal none of the findings characteristically seen in true atrophic arthritis.

3. From a laboratory standpoint findings that are usually noted in atrophic arthritis were absent, save for the presence of a quite active sedimentation test in each instance. Both cases revealed strongly positive agglutination, opsonocytophagic, and skin tests for brucellosis.

4. Each patient has responded quite satisfactorily to management for chronic brucellosis: one case, Mrs. R. S., as soon as she improved on treatment for chronic brucellosis, discontinued treatment. The condition, however, reappeared, and continuation of proper treatment has resulted in practical cure.

CONCLUSIONS

1. Two cases are here presented which are typically atrophic arthritis from a clinical standpoint.

2. Laboratory findings are presented which indicate that the arthritis was due to chronic brucellosis.

3. X-ray findings are set forth indicating that chronic brucellosis does not cause articular cartilage destruction nor epiphyseal bone involvement such as is in due time present in practically all cases of atrophic arthritis.

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MULTIPLE INVASION OF THE BLOOD STREAM*

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IT IS well known that bacteriologic study of infected tissue may disclose more than one organism. Kolle and Hebsch,[1] and Ford,[2] described many such cases and developed an elaborate classification. However, there has been no systematic study of multiple invasion of the blood stream. A few references have been made to cases of septicemia complicated by a secondary bacteriemia, but we have seen no report devoted to the subject of multiple blood stream infection.

In 1906 Libman[3] reported 98 cases of sepsis, among which were several instances of multiple infection. The combinations were: streptococcus + gonococcus, streptococcus + *B. proteus, B. coli* + streptococcus + *Micrococcus albus, Staphylococcus aureus* + *pyocyaneus,* and three cases with staphylococcus + streptococcus. Scott[4] reported a series of 80 patients with urologic disorders who had had routine blood cultures taken with each chill. They found five (6 per cent) who had more than one organism in the blood at the same time. The combinations were staphylococcus or streptococcus with either *B. proteus* or *B. pyocyaneus.* Neuhoff, Amfses, and Hirshfeld[5] reported a series of 150 patients with sepsis. In only one patient was there a multiple infection, a patient with both streptococci and staphylococci in the blood. Adams[6] reported a series of 12 patients with sepsis, two of whom had multiple blood invasion; in one patient an embalming wound resulted in a fatal septicemia with *Streptococcus hemolyticus* and *Vibrion septique;* and in another patient, incision of an area of cellulitis was followed by a fatal sepsis with staphylococcus and *Streptococcus hemolyticus.* Similar series were reported by Shanks and Khan,[7] Warren and Herrick,[8] Cadham,[9] Trask,[10] and by Doane and Cates.[11]

The following is a summary of the combinations described by the aforementioned authors:

Streptococcus + gonococcus	–	1
Streptococcus + *B. proteus*	–	1
Streptococcus + *B. coli* + *Micrococcus albus*	–	1
Staphylococcus + *B. pyocyaneus*	–	2
Streptococcus + staphylococcus	–	11
Streptococcus + *Vibrion septique*	–	1
B. typhosus + enterococci	–	5
Streptococcus + *B. typhosus*	–	1
Streptococci + *B. coli*	–	1
B. proteus + *B. coli*	–	1
Staphylococcus + *B. coli*	–	1
Staphylococcus + *B. influenzae*	–	1
Pneumococcus + influenzae	–	2
Staphylococcus + pneumococcus	–	2
Staphylococcus + pneumococcus + *B. coli*	–	1

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On the other hand, many authors present large series of septic patients where there is no mention made of multiple infections. These include Bean,[12] who reports 109 patients; Crocker, Valentine, and Brody,[13] who report 52 patients; Dennett[14] and Thelander,[15] who report, respectively, 115 and 32 cases in children; Glass,[16] 63 cases; and Kolmer,[17] 282 cases. This makes a total of 652 patients with sepsis in whom no instance of multiple infection is mentioned.

In this paper we are presenting 24 children who showed more than one organism in the blood stream during their period of hospitalization. These cases were collected over a five-year period and represent 8 per cent of all patients with bacteriemia studied during this time.

We found it best to classify these patients as follows:

(1) True mixed septicemia.
(2) Septicemia complicated by a secondary bacteriemia.
(3) Spurious mixed septicemia.
   a. Where the second organism is a variant of the first.
   b. Where a casual invader appears in the course of a sepsis.

METHOD

All patients with fever admitted to the pediatric wards between the years 1931 and 1935 had blood culture studies. The blood cultures were taken according to standard methods by an intern assigned to the service. All work was supervised by one individual, giving the results a good degree of uniformity throughout the years during which these cultures were taken. Fourteen cubic centimeters of the patient's blood were drawn and poured into culture media at the bedside. Five cubic centimeters were injected into each of two 200 c.c. Erlenmeyer flasks. The first flask contained 125 c.c. of veal infusion broth at pH 7.2; the second flask contained 125 c.c. of 2 per cent dextrose veal infusion broth of the same hydrogen-ion concentration. The remaining 4 c.c. were divided equally to form two pour plates, one with a 2 per cent dextrose veal infusion agar, and the other with a tube of plain veal infusion agar. Daily transplants were made from the two flasks, and smears were stained at the same time.

The invasion was considered "large" if there were more than ten colonies per cubic centimeter of blood. It was called "small" if only the liquid media showed growth. The size of invasion was "medium" if the broths were positive, but there were less than ten colonies on the plates.

I. TRUE MIXED SEPTICEMIA

By the term "true mixed septicemia" we refer to a type of sepsis where more than one organism is found in the same blood culture. There were nine patients in this group. The combinations of organisms were as follows:

1. *Streptococcus hemolyticus* + *B. coli*	4 cases
2. *Streptococcus hemolyticus* + *B. coli* + *Staphylococcus aureus*	1 case
3. *Streptococcus hemolyticus* + *Staphylococcus aureus*	2 cases
4. *Streptococcus hemolyticus* + Pneumococcus type XXI	1 case
5. *Staphylococcus aureus* + *B. coli*	1 case

The number of colonies isolated of each organism was recorded as "numerous" in five instances; "large" and "medium" in two; "small" in one, and "unknown" in one.

In six patients of this group the blood cultures were performed post mortem. These patients lived for such a short time, or were so desperately sick that a culture was not taken during life. In each instance a cardiac puncture was performed immediately after suspension of respiration, and the blood was poured into media at the bedside.

The significance of the blood cultures in these cases depends on what significance can be placed on post-mortem cultures. If the blood after death is rapidly invaded with many organisms which have no relation to the disease process, then these cases are of no practical significance. In our experience, however, such rapid nonspecific growth does not ordinarily occur. Of 1,309 patients from whom we cultured specimens over a period of five years, 171 had the blood drawn immediately after death. Of these, fully 50 per cent were sterile. We agree with White[18] in supposing that these post-mortem cultures reveal the etiologic agents of disease which cannot grow out during life, but multiply rapidly after death because the immunologic resistance of the body is no longer intact.

Most patients in this group were very young. Three patients were less than a month old, four were less than 6 months old, one was 3 years, and one was 6 years old. There were four girls and five boys. Short case histories of these nine patients are given, but certain facts in the clinical course are particularly striking. Six patients were admitted to the hospital in a markedly toxic state and died within twenty-four hours. The other three died within four days after admission. The majority suffered from debilitating illnesses before admission. Six had been sick for one month or more, and three had been ill for a period of two weeks. In two patients the septicemia followed an operation. One patient had had a herniotomy; the other had an abscessed kidney removed. The most constant symptom was diarrhea, which occurred in six of the nine patients. The case histories follow:

Case 1.—H. K., a 2-month-old male, was operated upon for an inguinal hernia because of mild obstructive symptoms for four days. He left the hospital on the tenth day following operation but returned two days later with marked diarrhea and dehydration. He died soon after readmission. Cardiac puncture immediately after death revealed 34 colonies per cubic centimeter of blood of *Streptococcus hemolyticus*, and 35 colonies of *B. coli*. Autopsy revealed partial atelectasis and empyema, lipoid pneumonia, parenchymatous degeneration of the liver, kidneys, and adrenals, and a well-healed operative scar.

Case 2.—M. S., a 3½-year-old girl, was admitted with acute lymphatic leucemia. One week after admission her blood culture was sterile, but ten days later she developed acute parotitis and died. Cardiac puncture immediately after death revealed innumerable colonies of both *Streptococcus hemolyticus* and *B. coli*. Autopsy report confirmed the clinical diagnosis.

Case 3.—G. T., a 6-month-old girl, was admitted with acute rhinopharyngitis, diarrhea, marked dehydration, and rickets. She seemed to improve for a few days with treatment, but at the end of the second week she developed cellulitis of the back and lapsed into acidosis and dehydration again. This time she did not rally and nine days later she died. Terminally, she developed the signs of a diffuse bronchopneumonia. Cardiac puncture immediately after death showed numerous colonies of *Streptococcus hemolyticus* and pneumococcus type XXI.

CASE 4.—I. B., a 3-week-old male infant, was admitted after having been ill for two days with acute rhinopharyngitis, marked cervical adenitis, and cellulitis of the neck. Four days later there was fluctuation in the area of cellulitis, and it was incised and drained. The child developed a marked diarrhea which persisted for three weeks. He then developed a diffuse bronchopneumonia and died. Blood culture on admission was sterile, but a cardiac puncture immediately post mortem showed numerous colonies of *Staphylococcus aureus* and *Streptococcus hemolyticus*.

CASE 5.—B. S., a premature female infant, was admitted from the nursery on the fifteenth day after birth, with an acute upper respiratory infection and diarrhea. Despite treatment the child grew steadily worse, developed signs of thrombosis in the cranial sinuses, and died. On admission the blood culture was sterile but cardiac culture immediately after death showed *Streptococcus hemolyticus* and *B. coli*. Autopsy revealed fetal atelectasis, passive congestion of the viscera, gastro-enteritis, focal pneumonia, focal necrosis of spleen and suprarenals, and thrombi in the cranial sinuses.

CASE 6.—L. R., a 3-month-old-male, was admitted with diarrhea and marked dehydration, after having been sick with an acute upper respiratory infection for two weeks. With treatment the dehydration seemed to improve, but the patient developed a cellulitis of the left hand, and a day later, gangrene of the right hand. Blood culture showed *Streptococcus hemolyticus* and *B. coli*. The patient died three days later. Autopsy showed cellulitis of the left hand, gangrene of the right hand, marked cerebral congestion, cloudy swelling of the kidneys, liver, and heart, and multiple abscesses of the lung and kidneys. Culture of these abscesses showed the presence of *Staphylococcus aureus* and *Streptococcus hemolyticus*.

CASE 7.—L. B., a 6-year-old girl, was admitted with a history of chills and fever for one week. On admission she was markedly delirious. There were numerous purpuric areas on her body. She died the next day. Culture on admission, twenty-four hours before death, showed many colonies of *Streptococcus hemolyticus* and a *Staphylococcus aureus* in the blood.

CASE 8.—E. F., a 4-month-old male infant, was admitted with a history of a hematuria and a loss of weight for a month. After study an operation was performed. The child's course was progressively downhill, and he died a month after admission. The child showed *Staphylococcus aureus* in the blood twice, and a small invasion with *Staphylococcus aureus* and *B. coli* once, following the first cystoscopy.

CASE 9.—I. M., a 13-day-old male child, who was perfectly normal for six days after birth, developed a severe diarrhea on the seventh day. On the eleventh day he was admitted to the pediatric service with dehydration and gastrointestinal intoxication. At this time, he also had a bilateral purulent acute otitis media and a definite rhinopharyngitis. A continuous intravenous solution of glucose and a blood transfusion were administered, but the patient grew steadily worse, and two days later died. A cardiac puncture was done immediately after death, and a culture of the blood showed innumerable colonies of *Streptococcus hemolyticus*, *B. coli*, and a *Staphylococcus aureus*. Autopsy revealed an acute enteritis, purulent otitis media, lobular pneumonia, and cloudy swelling of the viscera.

II. SEPTICEMIA COMPLICATED BY SECONDARY BACTERIEMIA

There were five patients in this group. In three instances the patient first had a *Staphylococcus aureus* sepsis and then developed a hemolytic streptococcemia. In one case of *B. coli* septicemia, a secondary invasion by *Streptococcus hemolyticus* occurred. The fifth was a patient who was admitted with hemolytic streptococcus sepsis and later developed *B. coli* septicemia.

In three cases the size of the original invasion was classed as "large," and in two it was designated as "medium." The number of colonies in the secondary invasion was "large" in four instances, and "small" in one.

Four of the five patients in this group were less than 6 weeks old. One was 18 months old. All five were boys. All died.

The incubation period varied from three to fourteen days. The shortest period of hospitalization was twenty-four days, and the longest period of hospitalization was fifty-five days. In three cases there was an afebrile interval between two septic periods. In these it appeared as though the patients were convalescing from the first blood stream invasion when the second invasion began. The duration of the first acute episode was from eleven to sixteen days, and of the second, from seven to eight days.

In three patients an extensive erysipelas or an erysipelas-like eruption heralded the appearance of the secondary streptococcemia. In one, the entire neck was involved. In another, the lesion spread over the entire chest. In the third, there was involvement of both shoulders and the scrotum. We hesitate to designate these diffuse, bright red, indurated skin infections as erysipelas, because in all instances there were large numbers of colonies of *Streptococcus hemolyticus* in the blood. In all of our cases of primary erysipelas, the blood culture was usually sterile or at most an occasional small, transitory invasion was found.

We could not find any valid factor to account for the onset of the secondary infection. Treatment of the original sepsis may have created new foci of infection. In all instances the primary sepsis was treated vigorously with blood transfusions, clyses, infusions, and in one case with staphylococcus toxoid. In addition, suppurative foci were incised. On the other hand, we have 315 cases of sepsis that received similar treatment without developing secondary bacteriemia. The case histories of the five patients in group II follow:

CASE 10.—A. S., a male, 3 weeks old, was admitted with a history of osteomyelitis of the right hip for ten days and of the right knee for two days. On admission he showed a diffuse pustular eruption and evidence of trauma to the scalp from a forceps delivery. Blood culture showed ten colonies of *Staphylococcus aureus* per cubic centimeter of blood. Some skin abscesses formed which required incision and drainage.

Supportive treatment was administered. The temperature subsided gradually and remained normal for a period of two weeks. The number of colonies in the blood stream gradually decreased, and after the third culture the blood was sterile. Toward the end of the third week after admission, there was a sudden elevation to 102° F.; swelling and redness of the left shoulder appeared at the same time. The shoulder was incised on the following day. Instead of a pure culture of *Staphylococcus aureus*, which had previously been found in all cultures of the skin lesions, there was now a mixture of staphylococcus and *Streptococcus hemolyticus*. The temperature, however, remained normal for four days; on the fifth day there was a rise, and the blood culture showed four colonies of *Streptococcus hemolyticus* per cubic centimeter of blood. The temperature continued to increase. Two days later there were 21 colonies of *Streptococcus hemolyticus* per cubic centimeter of blood, and on the tenth day after incision of the shoulder an erysipelas-like rash appeared over the shoulders and scrotum. Two days later the child died.

CASE 11.—B. H., a newborn baby boy, was well until the third day after birth, when he developed slight jaundice, fever, and a moderate diarrhea. On admission to the pediatric service on the sixth day of life, there were 35 colonies of *B. coli* per cubic centimeter; nine days later there was *B. coli* in one broth only. Moderate variations in the extent of invasion continued. One month after admission the culture was sterile and remained so for over a week. During this time the patient's clinical status seemed fair. At the end

of the fifth week there was a sudden rise in temperature to 104° F., and on the same day an erysipelas-like eruption appeared over the child's chest, and *Streptococcus hemolyticus* appeared in two broths. The rash spread, but the blood culture on the next day was still sterile. The child died four days later. Autopsy confirmed the diagnosis of erysipelas.

CASE 12.—N. E., an 18-month-old male baby, was admitted with a history of coryza and generalized anasarca of two weeks' duration. On admission, besides the edema, there was marked abdominal distention. Blood culture showed seven colonies of *Streptococcus hemolyticus* per cubic centimeter. Two days later the blood culture was sterile. On the sixth day an erysipeloid rash appeared over the lower back. The child had a septic temperature during the first two weeks, followed by an afebrile period of twenty days. At the end of that time he suddenly developed fever and diarrhea, and within twenty-four hours, intestinal intoxication. He died three days later. Cardiac puncture immediately after death showed innumerable colonies of *B. coli*.

CASE 13.—F. C., a male, 5 days old, was born at home after a difficult delivery. The infant was cyanotic and dyspneic for two days after birth. On the day of admission he became stuporous and gradually lapsed into coma. On examination a left Erb's palsy and evidence of intracranial injury was found. In addition there was a diffuse induration of the skin of the back. On the eleventh day after admission, the child's temperature became elevated, the edema of the back became red, and a mass appeared on the right buttock. Blood culture showed 20 colonies of *Staphylococcus aureus* per cubic centimeter. Two days later both abscesses were drained. The child's condition, however, did not improve, and six days later he died. Two days before death 50 colonies of hemolytic streptococci per cubic centimeter appeared in the blood.

CASE 14.—V. de P., an 8-day-old male, was admitted with jaundice and fever of a few hours' duration. On admission he was markedly jaundiced and somewhat cyanotic. A mild omphalitis was found. Blood culture showed *Staphylococcus aureus* in both broths. The child received supportive measures, but seven days later he still had 12 colonies of *Staphylococcus hemolyticus* in the blood. During the third week of his stay he developed evidence of a bronchopneumonia, but the blood culture was sterile. After this the course was steadily downhill. He developed diarrhea, and one week later cellulitis appeared at one of the transfusion sites, but this seemed to subside with hot compresses. Five days later an erysipeloid rash appeared on the scrotum and upper thighs. Blood culture showed 25 colonies of hemolytic streptococcus per cubic centimeter. The next day the infant died. Autopsy showed bronchopneumonia, cellulitis of scrotum and thigh, and acute lymphadenitis of nodes around the left common iliac vessels.

III. SPURIOUS MIXED SEPTICEMIA

In this group of patients the clinical features were determined by the primary rather than by the secondary invader.

The secondary invader was one of two types:

(A) Streptococcus gamma or viridans present in the blood as a casual invader, with no clinical or bacteriologic significance or

(B) The second organism was a variant of the first.

The bacteriologic details of group A are shown in Table I.

The details of the cases in group B are indicated in Table II.

Cases 20, 21, and 23 might also be in group A, but we felt that it was more suitable to place them in this group. In Cases 22 and 23 the so-called "degraded" form of the organism appeared in the blood cultures before the "virulent" form.

The clinical significance of these two types of secondary invaders is not known. In the first group (group A) we can see no more significance than we

see in the occasional culture of a gamma streptococcus in the blood of patients with an acute infectious disease. The mutation from the virulent to the non-virulent form of an organism makes the prognosis more favorable (Cases 19, 20, 21), whereas the reverse usually spells a fatal outcome (Cases 22, 23).

TABLE I

CASE NO.	PRIMARY ORGANISM	SIZE OF INVASION	CASUAL INVADER	NO COLONIES	OUTCOME
15	*Staphylococcus albus* hemolyticus	Medium	Streptococcus gamma	Small	Recovered
16	*B. coli*	Large	*Streptococcus viridans*	Small	Died
17	*B. coli*	Unknown	Streptococcus gamma	Small	Died
18	*Staphylococcus*	Large	Streptococcus gamma		Died

TABLE II

CASE NO.	PRIMARY ORGANISM	SIZE OF INVASION	SECONDARY INVASION	SIZE OF INVASION	OUTCOME
19	*Staphylococcus aureus hemolyticus*	Medium	*Staphylococcus aureus* nonhemolyticus	Medium	Recovered
20	*Streptococcus hemolyticus*	Small	*Streptococcus viridans*	Small	Recovered
21	*Streptococcus hemolyticus*	Medium	Streptococcus gamma	Small	Recovered
22	*Streptococcus viridans*	Small	*Streptococcus hemolyticus*	Large	Died
23	*Streptococcus viridans*	Small	Pneumococcus XX	Medium	Recovered
24	*Dysentery-like organism*	Unknown	*B. coli*	Unknown	Died

CONCLUSION AND SUMMARY

In 8 per cent of a total of 320 cases of septicemia, a second organism appeared in the blood stream at some time during the illness. In 3 per cent, two organisms of definite pathogenicity appeared in the same culture. In 1.5 per cent invasion by the second organism appeared as a complication of the original sepsis, often when the first infection seemed to be on the decline. In the remaining cases (3.5 per cent) the second invader seemed to have no clinical or pathologic significance, but appeared merely as a casual invader of the blood stream or as a variant of the first organism.

One might suppose that once the body resistance to blood stream invasion is overcome, any number of organisms could follow the first invader. If we may look upon sepsis as an infectious disease, we see that septicemia is not complicated by a superimposed blood stream invasion any more often than other infectious diseases. It would appear that the immunologic changes which permit the onset of a sepsis are specific for one organism. Invasion by more than one organism

rarely takes place. When it does occur, it is seen only in very young infants and the outcome is invariably fatal.

Two points of special clinical interest are worthy of note. The first is the appearance of an erysipeloid rash when a hemolytic streptococcemia is superimposed on an existing sepsis. The other is the difference in the clinical course in types I and II. In the cases with two organisms in the same blood culture the incubation period was long, followed by a short, acute course terminating rapidly in death. Where the second invasion complicated a primary sepsis, the incubation period was short. The illness was characterized by two septic periods separated by an afebrile period, the second coincidental with the second invasion.

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OBSERVATIONS ON THE TOXICITIES AND CHOLERETIC ACTIVITIES OF CERTAIN BILE SALTS*

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IT IS a well-known fact that the toxicity of bile is due to its bile salt content.[1-6] The choleretic properties of bile salts are also known.[7-13] Comparisons of the relative toxicities and choleretic activities of the various bile salts have been limited by the fact that many of them have not been available in pure form. Some of the contradictory results which have been reported may be attributed to the fact that preparations of different degrees of purity have been used. We have recently had available a number of highly purified bile acid preparations and have studied their toxicity and choleretic activity in an attempt to correlate these properties with chemical structure.

METHODS

The toxicities of seven bile salts have been compared by blood pressure studies on anesthetized dogs and by in vitro hemolysis studies on human erythrocytes. The relative choleretic activities have also been studied in the anesthetized dog.

Bile Salt Preparations.—The sodium salts of cholic acid, dehydrodeoxycholic acid, apocholic acid, and deoxycholic acid were prepared by dissolving the pure bile acids† in alcohol, neutralizing with dilute sodium hydroxide to the phenolphthalein end point, evaporating to dryness, and finally dissolving the dry salt in distilled water. Dehydrocholic acid† was furnished us as the sodium salt. We have used two different preparations of sodium glycocholate and of sodium taurocholate, one a natural product† and one a synthetic product.‡ Aqueous solutions of the natural products were yellow in color whereas the synthetic salts gave clear colorless solutions.

Chemical assays revealed a 56 per cent cholic acid content for the natural taurocholate, corresponding to 71 per cent purity; the synthetic taurocholate assayed 80 per cent cholic acid, corresponding to 100 per cent purity; the synthetic glycocholate assayed 87 per cent cholic acid, corresponding to 97 per cent purity.

Blood Pressure Studies.—Dogs under sodium pentobarbital anesthesia were used. A tracheal cannula was inserted, and the carotid blood pressure was recorded. The common bile duct was cannulated close to the duodenum, and the

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cystic duct was ligated. The sodium salts of the bile acids in 10 per cent solution were used. All injections were given into the femoral vein at a standard rate (fifteen seconds). The doses arbitrarily chosen were 30 mg. per kilogram, which is the standard intravenous dose of sodium dehydrocholate in man, 10 mg. per kilogram, and 5 mg. per kilogram. Several injections were given to the same animal. At least twenty to thirty minutes were allowed between each injection, which was ample time for the blood pressure to return to normal. The order in which the different salts were given was varied in each animal.

Hemolysis Studies.—Hemolytic activity was studied by the method of Ponder[14] using human cells. Solutions containing 50 mg. of bile salt in 4 c.c. of 0.9 per cent sodium chloride solution were made up. Dilutions of this solution were then prepared: 1:10, 1:100, 1:1,000, etc. One cubic centimeter of standard suspension of human red blood cells was added to 4 c.c. of bile salt solution, and the time for complete hemolysis was noted. The tests were made in a water bath at 38° C.

Choleretic Studies.—The dogs were prepared as described for the blood pressure studies. After a uniform bile flow was established, a control period of thirty minutes to one hour was recorded. Thirty milligrams per kilogram of bile salt in 10 per cent solution was then injected into the femoral vein, and the bile volume was recorded every fifteen minutes until the rate of secretion had returned to the control level.

RESULTS

Blood Pressure Studies.—The averaged results of the blood pressure studies are given in Table I. A 10 mg. per kilogram dose of each of six salts was tested on 10 animals. The 30 mg. per kilogram dose was tested on 10 animals with four salts and on 5 animals with two. Because of the limited supply of sodium taurocholate available, only a single dose, 30 mg. per kilogram, was tested on 6 animals. A 30 mg. per kilogram dose of synthetic sodium glycocholate was tested in 6 experiments on 3 animals. In 4 animals a 5 mg. per kilogram dose of apocholate and deoxycholate was tested.

As indicated in Table I, sodium dehydrocholate was the least toxic and sodium deoxycholate was the most toxic of the seven salts tested. There appears to be little difference between the conjugated salts, cholate, and dehydrodeoxycholate, although the latter seems to be somewhat more toxic. There was no significant difference between the effect of the synthetic and natural preparations of the glycocholate and taurocholate. In 4 animals 5 mg. per kilogram of apocholate produced an average depression of 17 mm. Hg, and deoxycholate produced 21 mm. Hg. These depressions are greater than those produced by twice the dose of the other four salts in which a 10 mg. dose was tested, and by three times the dose of dehydrocholate. In summary, the salts may be divided into three groups. Group I contains only one salt, dehydrocholate, which is by far the least toxic. Group II contains four salts of intermediate toxicity, cholate, two conjugated salts, and dehydrodeoxycholate. Finally, Group III contains the two most toxic salts, apocholate and deoxycholate.

TABLE I

DEPRESSOR ACTION OF BILE SALTS

SALT	DOSE (MG./KG.)	NO. OF DOGS	BLOOD PRESSURE—MM. OF HG.		
			CONTROL	MINIMUM	MILLIMETERS CHANGE
Dehydrocholate	10	10	144	143	- 1
Cholate	10	10	146	138	- 8
Glycocholate (N)	10	10	149	138	-11
Dehydrodeoxycholate	10	10	161	151	-10
Apocholate	10	10	152	124	-28
Deoxycholate	10	10	158	125	-33
Dehydrocholate	30	10	144	134	-10
Cholate	30	10	140	112	-28
Taurocholate (S)	30	3	119	95	-24
Taurocholate (N)	30	3	109	84	-25
Glycocholate (S)	30	3 (6 tests)	131	103	-28
Glycocholate (N)	30	10	135	103	-32
Dehydrodeoxycholate	30	10	143	102	-41
Apocholate	30	5	152	87	-65
Deoxycholate	30	5	152	79	-73
Apocholate	5	4	124	107	-17
Deoxycholate	5	4	117	96	-21

Comment.—Selecting cholic acid as the parent compound, the effects of various changes in this basic structure may be examined. Oxidation of the three hydroxyl groups of cholic acid to ketone groups markedly decreases the toxicity. Conjugation of cholic acid with glycine or taurine does not have a marked effect on the toxicity. The removal of the hydroxyl group on C7 of cholic acid very markedly increases toxicity. *Oxidation of the two hydroxyl groups of deoxycholic acid markedly (44 per cent) decreases the toxic properties of the latter compound,* though not so markedly (64 per cent) as the oxidation of cholic acid. Apocholic acid, which differs from deoxycholic only in having one ethylene linkage (C8 = C9), has toxic properties similar to those of the saturated substance.

Hemolysis Studies.—Difficulty was experienced in obtaining cell suspensions with uniform properties, even though the blood was always taken from the same person and an exactly similar technique was employed in the preparation. However, although the absolute dilutions did not check exactly with different cell suspensions, the order of hemolytic activity of the different salts tested on the same cell suspension was always the same. On the basis of our results the various salts may be divided into three groups according to their hemolytic activity (Table II).

TABLE II

HEMOLYSIS BY BILE SALTS

Group I —Weakly hemolytic	1. Dehydrocholate
Group II —Moderately hemolytic	1. Synthetic glycocholate
	2. Synthetic taurocholate
	3. Cholate
	4. Dehydrodeoxycholate
Group III—Strongly hemolytic	1. Natural glycocholic
	2. Natural taurocholic
	3. Apocholic
	4. Deoxycholic

Comment.—Oxidation of the three hydroxyl groups of cholic acid decreases the hemolytic activity of this substance. The removal of the OH group on C7 markedly increases the hemolytic activity. The hemolytic properties of deoxycholic acid are decreased by oxidation to the ketoacid. The conflicting results obtained with the conjugated salts are of interest. The synthetic products were only weakly hemolytic, being second only to the dehydrocholate. On the other hand, the natural products were strongly hemolytic. It is probable that impurities (fatty acids) in the natural products were responsible for the discrepancy. If the synthetic products represent the action of the pure salts, then conjugation does not markedly affect the hemolytic properties of cholic acid.

TABLE III

CHOLERETIC EFFECTS OF BILE SALTS

(30 Mg. per kilogram of body weight intravenously)

SALT	NO. OF ANIMALS	BILE VOLUME PER 1 HOUR PERIOD			30 MIN. AFTER INJECTION
		CONTROL*	FIRST HOUR AFTER INJECTION	% CHANGE	
1. Dehydrocholate	5	2.0	13.5	+575	16.8
2. Apocholate	5	2.0	10.0	+400	11.8
3. Deoxycholate	6	2.0	8.9	+345	11.2
4. Cholate	6	2.0	7.3	+265	9.3
5. Dehydrodeoxycholate	5	2.0	6.4	+220	8.3
6. Glycocholate (S)†	6	2.0	6.4	+220	7.2
7. Taurocholate (S)†	9	2.0	5.5	+175	6.9

*In order to illustrate the relative choleretic effects more clearly, all of the volume outputs have been recalculated on the basis of a common control value, i.e., 2 c.c. per hour. The actual control values varied from 1.6 to 2.6 c.c. per hour.

†Sufficient of the natural compound was not available for this study.

The first three salts produced a choleresis lasting somewhat longer than the last four; the choleresis of the last four was completed in one to one and one-half hours.

Choleretic Studies.—The results of the choleretic studies are summarized in Table III. Sodium dehydrocholate is by far the best choleretic. It causes a greater and more prolonged choleresis than any of the other salts studied. Based on the percentage increase in bile output during the first hour after injection, the salts may be grouped as follows in the order of decreasing choleretic properties:

Group I (greatest choleresis)	Dehydrocholate
Group II	Apocholate Deoxycholate
Group III	Cholate Dehydrodeoxycholate Glycocholate (S)
Group IV	Taurocholate (S)

Comment.—Oxidation of the three hydroxyl groups of cholic acid to keto groups markedly increases the choleretic properties. It also appears that removal of the hydroxyl group on C7 of cholic acid increases the choleretic activity somewhat. Preliminary results on chronic biliary fistula dogs[15] do not support this finding. In these experiments the bile salts were given orally, and deoxycholic acid proved to be only about two-thirds as effective a choleretic as

cholic acid. On the other hand, it has been reported that in the anesthetized rabbit, apocholic acid produces a greater choleresis than dehydrocholic acid,[13] and that both apocholic and deoxycholic acid produce a greater choleresis than cholic acid.[10] The results of recent studies[16] suggest that conjugation suppresses the choleretic effect of the ketocholanic acids. The authors of this report point out the fact that their results do not mean that conjugation of all ketocholanic acids will depress their choleretic activity or that the oxidation of all cholanic acids will give them hydrocholeretic properties.

Our results show that equivalent weights of the conjugated acids are less choleretic than cholic acid. Assuming choleresis to be dependent on cholic acid content, and using the choleresis it caused as a base, one would expect approximately a 213 per cent choleresis with taurocholic acid and a 234 per cent choleresis with glycocholic acid (since our taurocholic acid preparation assayed 80 per cent cholic acid and our glycocholic 87 per cent cholic acid). Our results thus appear to indicate that conjugation with glycine has little effect on the choleretic properties of cholic acid, whereas conjugation with taurine depresses this property. Preliminary results on chronic fistula dogs also appear to indicate that conjugation with taurine depresses the choleretic action of cholic acid.

It is of interest that oxidation of deoxycholic acid to the dehydro form did not in our experience increase the choleretic properties of this substance. Adlersberg and Lustig[13] quote unpublished work which indicates that dehydrodeoxycholic acid is less toxic and more choleretic than the hydroxyacid. They also report acute experiments in rabbits showing that dehydroapocholic acid is more choleretic than apocholic, and that both the latter acids are better choleretics than dehydrocholic acid. Sobotka[17] states that dehydrodeoxycholic acid is a better choleretic than dehydrocholic. The source of this statement is not given. In our experience, dehydrocholic acid was by far the best choleretic.

DISCUSSION

The literature on the toxicity of bile has been completely reviewed in Horrall's recent book.[6] Much of the earlier work is of questionable significance because pure substances were not used. To summarize an extensive literature, toxicity studies by various methods (lethal doses in different animals, effect on excised heart and skeletal muscles, effect on the gall bladder) reveal that cholic acid is much less toxic than deoxycholic and that dehydrocholic acid is the least toxic of all. The majority of studies on hemolysis place the order of increasing hemolytic activity as follows: dehydrocholic < cholic < apocholic < deoxycholic. In general, these results are in agreement with our own. It is worthy of note that the relative toxicity, as tested by depression of blood pressure and hemolysis, is practically identical. The structural implications of our results have already been discussed.

The majority of the pertinent literature on choleresis has been cited. Regan and Horrall[11] found glycocholic acid to be more toxic and less choleretic than dehydrocholic acid. This is in agreement with our findings.

SUMMARY AND CONCLUSIONS

1. The toxic and choleretic properties of seven bile salts have been studied in the dog.

2. Based on depressor and hemolytic activity, dehydrocholate is the least toxic salt, and apocholate and deoxycholate are the most toxic salts. The other four salts form a group of intermediate toxicity.

3. A discrepancy in the hemolytic activity of two different preparations, one synthetic and the other a "natural" but not pure, of the conjugated cholates has been noted. The probable explanation of this fact is discussed.

4. Sodium dehydrocholate, or oxidized cholic acid, or a ketocholanic acid, is the most effective choleretic, as well as the least toxic. Sodium taurocholate is the least effective. Deoxycholic acid is not a good choleretic and is relatively quite toxic.

5. The influence of chemical structure on the toxic and choleretic properties of the bile acids has been discussed.

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MULTIPLE SERUM ALLERGY IN ATOPIC INDIVIDUALS*

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WITH the increasing use of animal sera in modern therapeutics, the subject of serum allergy is assuming ever increasing importance. It is not the purpose of this paper to undertake a general discussion of this subject,† but rather to determine the frequency with which multiple serum allergy occurs in allergic individuals, and the possible dangers involved when serum of one animal is substituted for that of another without preliminary tests.

Serum allergy may be defined as a state of hypersensitivity to a foreign serum, usually the result of a previous serum injection. In the allergic patient it may be coincident with a sensitiveness to the animal dander which contains also a small amount of serum antigen. The parenteral administration of a foreign serum in atopic persons even in minute quantities, is likely to result in serum reactions. The latter may be of the delayed type—accelerated serum sickness, or the precipitating or immediate reaction. A history of asthmatic attacks when in contact with horses, "horse asthma," or, for that matter, a history of allergic manifestations when in contact with other animals, increases the possibility of severe or even fatal reactions to a much greater degree. Boughton[1] in 1919 reported a death which resulted from the administration of one minim of horse serum intravenously to a horse asthmatic. Fatal reaction from an intracutaneous test with 0.05 c.c. of horse serum was described by Friedman[2] in 1935. Lobo[3] in 1936 reported a death which resulted from the subcutaneous injection of 5 c.c. of antidysentery serum to a 1-year-old child with "exudative diathesis." In 1937 Vaughan and Pipes,[4] in reviewing the literature from 1923 to 1935, found reports of 35 fatalities from serum administrations, 6 of the deaths following intracutaneous tests with undiluted serum. Shock following an intravenous test[7]‡ with a 1:50 dilution of rabbit serum in two allergic individuals was described by Horsfall and his co-workers.[5]

As the reports in the literature deal mostly with horse serum, the question arises whether the dangers involved in its use in allergic persons may be circumvented by substituting a serum obtained from an animal other than the horse. Horsfall and his co-workers,[5-7] for example, have advocated the substitution of antipneumococcus serum obtained from the rabbit for that of the horse. They set forth the many desirable advantages that it offers immunologically and also

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†For a comprehensive analysis of the literature on serum allergy, the reader is referred to a review by Harten and Walzer, J. Allergy **11**: 68, 1939.

‡These authors feel that the intravenous test to establish serum sensitivity in allergic persons, because of the possible occurrence of severe immediate serum reactions or even fatalities, is a procedure fraught with much danger and should, therefore, not be used routinely, if at all.

pointed out that a smaller percentage of persons are apt to be sensitive to rabbit serum than to horse serum. That such is the case cannot be denied, considering the fact that immune sera up to recent times have been derived from the horse almost exclusively. One must bear in mind, however, that the occurrence of atopic serum allergy does not necessarily require a previous serum injection, as exposure to the animal dander in allergic persons may sensitize them to the homologous serum as well. That horse dander contains also a small amount of horse serum antigen has been amply demonstrated by the work of Rackemann,[8] Forster,[9] Ratner and Gruehl,[10] and Tuft.[11] That this is also true of other animal danders may be deduced from the work of Walker,[12] who showed that 70 per cent of his cat dander cases were also allergic to cat serum. These figures indicate an even higher percentage of serum antigen in the danders of these animals than in that of the horse, since he obtained only 22 per cent of horse serum reactions in those allergic to the horse dander.

Simon[13] in 1934 reported 12 patients whom he skin-tested with 9 mammalian sera and found that 3 of these reacted to all 9 sera, 4 reacted to horse serum plus one or two additional sera, and 5 were sensitive to horse serum alone. As a result of these findings, he concluded that there must be a common serum antigen in all mammalian sera in addition to a species-specific antigen characteristic of each one; and that whereas most people become sensitized to the latter factor, some few become allergic to the common factor. Two of 67 patients with pneumonia reported by Horsfall and his co-workers[7] were found to be sensitive to horse serum and went into shock after an intravenous test with rabbit serum, thus indicating that they were sensitive to at least two sera.

It appears, therefore, from these observations that there are persons who may be sensitive to more than one serum. Indeed, some probably are sensitive to all mammalian sera. In view of these facts it was our object to investigate: (1) the frequency with which sensitivity occurs in allergic persons to the serum of horse and other animals; (2) the value of the skin and ophthalmic tests in the diagnosis of serum allergy; (3) the relationship between serum and dander allergy; and (4) the comparative incidence of horse serum allergy in allergic persons who previously had received serum injections and in those who had not.

Forty patients from our Allergy Clinic, consisting of hay fever and asthma cases, were tested with 1:100 and 1:10 dilutions of horse, goat,* rabbit,* and sheep serum with the intracutaneous skin and ophthalmic tests, and also with the corresponding danders (Table I). Of these, 3 cases originally reacted to 1:100 dilution of horse serum by the intracutaneous skin test with a four-plus† reaction. These cases and any others that reacted to the higher dilution of a serum were, for obvious reasons, not tested with the 1:10 dilution. If the reaction with the 1:100 dilution was three plus or less, a 1:10 dilution of the 4

*Rabbit and goat sera were kindly supplied by the Lederle Laboratories.

†The reactions were read after an interval of ten to fifteen minutes, according to the following scheme:

Four plus—large wheal 2 cm. in diameter or larger, with definite pseudopodia.
Three plus—wheal smaller than above, with no or slight pseudopodia.
Two plus—definite wheal smaller than above, no pseudopodia.
One plus—erythematous flare with slight or no wheal.
0—no discernible reaction.

different sera was used for skin testing. On a subsequent visit,* conjunctival tests† were performed with 1:100 dilution of the sera. If the test was positive, the higher concentration was not used. When the opposite was the case, the tests were repeated with the 1:10 dilutions. No more than one conjunctival test was done at one time; the other eye was used as a control. In case of obvious lack of sensitivity more than one serum was used for testing after a proper interval.

Fig. 1.—Case 1. Patient S. B. Direct intracutaneous skin tests with 4 different sera diluted 1:100. Photographed after twenty minutes. All reactions were markedly positive to the 4 sera, showing wheal with pseudopods and area of erythema. *a*, Horse serum 1:100; *b*, goat serum 1:100; *c*, rabbit serum 1:100; *d*, sheep serum 1:100; *e*, control, buffered saline; *f*, control, buffered saline.

Of the 40 allergic individuals studied, 3, or 7.5 per cent, yielded four-plus skin and positive conjunctival reactions to all 4 sera (Table II; Figs. 1 and 2). These 3 cases in addition exhibited systemic reactions besides the marked local reactions. The former consisted of sneezing fits and attacks of asthma which required the administration of epinephrine. One patient (Case 1) also exhibited angioneurotic edema of eyelids and face, and generalized urticaria; another (Case 24) showed four-plus skin reactions to goat and sheep sera, and negative reactions to the other two sera; the ophthalmic reactions were not done due to the patient's failure to return to the clinic. Four patients, or 10 per cent, evidenced four-plus skin reactions to one or more serum, 3 of them also exhibited positive conjunctival tests, 1 was not tested; the remaining 36 cases evidenced skin reactions varying from three to one plus; and none gave a positive eye test. If all degrees of skin reactions are considered, then there were 25 cases who reacted to horse serum, 21 to goat serum, 21 to rabbit serum, and 22 to sheep serum. Completely negative reactions were obtained in the remainder (Table II). The number of four-plus skin reactions obtained by Brown and Seehzer[14]

*It was found wise to perform the intracutaneous and ophthalmic tests on different days, since positive reactions to both were followed by constitutional reactions in 3 cases, necessitating the use of adrenalin, and, in one case, the use of aminophyllin also.

†A conjunctival test was considered positive if there occurred a definite redness of the palpebral and bulbar conjunctivae due to the injection of the conjunctival vessels associated with itching and lacrimation within five to ten minutes after the instillation of the test solution into the conjunctival sac. In case of marked reactions systemic manifestations also occurred.

TABLE

RESULTS OF INTRACUTANEOUS AND OPHTHALMIC

CASE	AGE	SEX	ALLERGY ON CONTACT WITH ANIMALS	HISTORY OF PREVIOUS SERUM INJECTIONS	HISTORY OF PREVIOUS SERUM SICKNESS	HORSE					
						SERUM				DANDER	
						INTRACUTANEOUS		OPHTHALMIC			
						1-100	1-10	1-100	1-10	100	10
1.—S. B.	20	F	No	D.A.T.*	No	++++		++++		++++	
2.—N. B.	9½	F	No	No	No	0	0	0	0	0	0
3.—N. B.	9½	M	No	T.A.T.†	No	0	++	0	0	0	0
4.—M. C.	50	F	No	No	No	0	0	0	0	++	+++
5.—R. D.	32	F	No	D.A.T. at age of 12	No	0	0	0	0	0	0
6.—I. E.	30	M	No	T.A.T.	No	0	+	0	0	0	0
7.—N. E.	12	M	No	No	No	0	0	0	0	+	+
8.—W. G.	22	M	B.A.‡ Rabbit wool?	No	No	++++		++++			++
9.—H. G.	13	F	No	D.T.A.T.?‡	No	++	+++	0	0	0	0
10.—R. K.	30	M	No	D.T.A.T.—twice last 18 mo.	No	0	0	0	0	0	0
11.—Y. K.	15	F	No	T.A.T.—twice 2 and 3 yr. ago	No	0	0	0	0	0	0
12.—F. L.	34	F	No	No	No	0	++	0	0	0	+
13.—L. M.	42	F	No	No	No	0	0	0	0	0	0
14.—C. M.	56	F	No	No	No	++	++	0	0	++	+++
15.—S. P.	14	F	No	No	No	++++		++++			++++
16.—R. P.	6	M	No	No	No	0	0	0	0	0	0
17.—J. S.	41	F	No	No	No	+	+	0	0	0	0
18.—I. I.	42	F	No	No	No	0	+	0	0	0	+
19.—L. W.	14	M	No	D.T.A.T.	No	0	0	0	0	0	0
20.—M. W.	41	M	No	No	No	0	+	0	0	0	0
21.—M. Z.	55	F	B.A.‡ Horses	No	No	+	+	0	0	++++	0
22.—A. P.	51	M	No	No	No	+	+	0	0	0	0
23.—M. H.	50	M	No	No	No	0	+	0	0	0	0
24.—P. G.	32	F	No	No	No	0	0	0	0	0	0
25.—J. W.	28	M	No	No	No	+	+	0	0	0	++
26.—B. F.	55	M	No	No	No	0	0	0	0	0	0
27.—S. F.	32	F	No	No	No	0	+	0	0	0	0
28.—P. G.	60	M	No	No	No	+	++	0	0	+	++
29.—A. H.	53	M	No	No	No	0	0	0	0	0	0
30.—P. S.	11	M	No	T.A.T.—twice at age 3	No	++	+++	0	0	0	0
31.—J. C.	8	M	No	T.A.T.—twice at age 7	No	0	+	0	0	0	0
32.—A. R.	54	M	No	No	No	0	0	0	0	0	0
33.—R. B.	10	F	No	D.T.A.T.	No	0	0	0	0	0	0
34.—I. B.	40	M	No	No	No	0	+	0	0	0	0
35.—A. N.	54	M	No	No	No	0	+	0	0	+	++
36.—E. G.	32	F	No	D.A.T.	No	0	++++		++++		++++
37.—P. R.	33	F	No	No	No	0	++	0	0	+	++
38.—F. G.	36	F	No	No	No	+	++	0	0	0	0
39.—S. B.	38	M	No	No	No	0	0	0	0	0	0
40.—R. F.	41	F	No	No	No	+	+	0	0	0	0

*D.A.T.—Diphtheria antitoxin.
†T.A.T.—Tetanus antitoxin.
‡B.A.—Attack of bronchial asthma.

I

TESTS WITH 4 FOREIGN SERA ON 40 ALLERGICS

GOAT						RABBIT						SHEEP					
SERUM				DANDER		SERUM				DANDER		SERUM				DANDER	
INTRACUTANEOUS		OPHTHALMIC				INTRACUTANEOUS		OPHTHALMIC				INTRACUTANEOUS		OPHTHALMIC			
1-100	1-10	1-100	1-10	100	10	1-100	1-10	1-100	1-10	100	10	1-100	1-10	1-100	1-10	100	10
++++		++++			+	++++		++++		+	+	++++		++++		+	+
0	0	0	0	0	0	0	0	0	0	0	0	0	+				
0	0	0	0	0	0	0	0	0	0	0	+	+	++	0	0	0	0
0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	+	0	0	0	0	+	++	0	0	0	0
0	+	0	0	0	0	0	+	0	0	0	+	0	+	0	0	0	++
0	0	0	0	0	0	0	+	0	0	0	0	0	+	+	0	0	++
++++		++++			++++	++++		++++		++++		++++		++++			+
+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	+	0	0	0	0	+	0	0	0	0	0	++	0	0	0	0
0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0
0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0
0	+	0	0	0	+++	+	++	0	0	0	+	+	+	0	0	0	0
++++		++++		0	0	+++	++++	++++			++++	++++		++++			+
0	++	0	0	++	++++	0	++	0	0	0	0	0	0	0	0	0	+
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++
0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+
0	+	0	0	0	0	0	++	0	0	0	0	0	++	0	0	0	++
0	++	0	0	0	0	+	+	0	0	0	0	+	+	0	0	0	++
+	++	0	0	0	0	0	+	0	0	0	+	0	+	0	0	0	0
0	+	0	0	0	0	+	++	0	0	0	0	+	++	0	0	0	0
0	+	0	0	0	0	0	+	0	0	0	0	+	++	0	0	0	0
	++++	Not done			0	0	0	Not done		0	0		++++	Not done		0	0
+	+	0	0	+	+	+	+	0	0	0	0	+	+	0	0	+	+
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+	+	0	0	0	0	+	+	0	0	0	0	+	+	0	0	0	0
0	0	0	0	+	++	0	+	0	0	0	0	+	++	0	0	0	+
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	+	++	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	+	0	++	0	0	0	0	0	0	0	0	0
+	++	0	0	+	++	0	0	0	0	0	+	0	+	0	0	0	+++
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	+	+	++	0	0	++	++	+	++	0	0	0	0
0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+	+	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0

with rabbit serum tests in 101 allergic persons were 2 as compared with 3 such reactions in our series of 40 cases; we obtained 3 positive conjunctival tests to their 1. The discrepancy in these figures may be explained by the fact that these authors tested 101 cases at random, whereas we started with 3 patients who were allergic to horse serum.

Fig. 2.—Case 1. Patient S. B. Positive ophthalmic test of right eye (one drop of 1:100 sheep serum was instilled); left eye control. Photographed after ten minutes. Reaction, however, started in about three minutes. Patient experienced marked itching and also tearing of the eye. Rhinorrhea, sneezing, and choking of the chest with dyspnea also occurred. Adrenalin 1:1,000 locally and subcutaneously had to be administered.

Brief case histories of 5 persons who reacted positively to one or more serum and one or more danders are given.

CASE REPORTS

CASE 1.—S. B., a female stenographer, aged 20 years, had perennial bronchial asthma. She gave no history of allergic symptoms on contact with animals, nor of any previous serum administration. She reacted to all 4 sera both by skin and eye tests. Four-plus skin reactions were also obtained with horse and cat dander and pork, beef, and lamb. There were clinical improvements from the elimination of these foods. She exhibited constitutional reactions following skin tests with all 4 sera, conjunctival test with one of them necessitating the use of adrenalin and aminophyllin for the relief of asthma and angioneurotic edema (Figs. 1 and 2).

CASE 8.—W. G., a male, aged 22 years, waiter at the Hospital, had perennial asthma. He gave a definite history of asthmatic attacks when in proximity to rabbits and when working in a sweater factory. There was no history of any serum injections. He reacted to all 4 sera both by skin and conjunctival tests, and also showed four-plus skin reactions to goat, rabbit, cat and dog epithelia, and also to pork, lamb, beef, and milk. Constitutional reaction resulted from the positive skin and eye tests, necessitating the use of adrenalin.

CASE 15.—S. P., a 14-year-old schoolgirl, had perennial asthma. She had had diphtheria toxin-antitoxin in childhood, but no other sera. She gave no history of any allergic manifestations on contact with animals. Four-plus skin reactions to all 4 sera, as well as positive conjunctival tests, were obtained. The danders of horse, rabbit, cat, and dog, as well as pork, lamb, and beef, yielded four-plus skin reactions. Constitutional reaction resulted from the positive skin and eye tests, necessitating the use of adrenalin.

CASE 24.—P. G., an Austrian female, aged 32 years, domestic, had seasonal hay fever and was asthma sensitive to grasses. She presented no history of any prior serum administration

nor allergy on contact with animals. Goat and sheep sera yielded positive four-plus skin reactions. Horse and rabbit sera yielded negative reactions. The ophthalmic reactions were not done since the patient failed to return. No reactions to the homologous or to other danders were obtained.

CASE 36.—E. G., a female, aged 32 years, housewife, had seasonal hay fever and was asthma sensitive to ragweed and trees. She had diphtheria antitoxin in childhood, but no other sera. She gave no history of allergic symptoms on contact with animals. She had a four-plus skin and positive conjunctival test to horse serum. She did not react to the other sera. She also exhibited a four-plus skin reaction to horse dander but not to other danders.

In studying the case histories of patients who showed positive reactions to serum, it may be noted that they also reacted to one or more animal danders. It has already been mentioned earlier in this paper that animal danders also contain a small amount of the homologous serum antigen. Exposure, therefore, to the animal dander may well sensitize an allergic individual to the serum. This may serve to explain, as brought out by Ratner and Gruehl's[10] experiments on guinea pigs, the existence of allergy to serum in atopic persons in the absence of previous serum injections. Of the 3 patients who evidenced four-plus skin and positive eye reactions to all 4 sera, one (Case 8) reacted with four-plus skin reactions also to goat and rabbit danders; another one (Case 1) evidenced similar reactions to horse dander; another (Case 15) to horse and rabbit danders. The one (Case 24) that reacted only to goat and sheep sera did not exhibit any positive reactions to dander, and the one (Case 36) who was positive to horse serum only also reacted to horse dander. Although the number of cases with positive dander reactions* (4 plus) are too small in this series to warrant arriving at any conclusions, it may nevertheless be of interest to note that in atopic persons who are sensitive to animal danders, there is a tendency for them to be also allergic to the homologous serum. The 4 patients with four-plus reaction to horse epithelium were also positive to the homologous serum by skin and eye tests; one case out of 2 with four-plus reaction to the goat dander was also allergic to the serum; both cases with four-plus reaction to rabbit dander also reacted to the serum. Not one out of 4 positive sheep dander cases reacted to the serum.

Tuft[15] in 1932 in a study of serum allergy after diphtheria toxin-antitoxin administration found that those who gave positive reactions showed a positive family or personal history of allergy in 34 per cent of cases, as compared with 7 per cent in controls. These findings, he states, show that the "allergic constitution is an important factor in predisposing these individuals to acquired serum allergy." He also offers evidence to prove that this may not be mediated through the reagin mechanism. In our group of 40 allergies, as far as could be determined by questioning, only 12 had previously received either diphtheria toxin-antitoxin, or diphtheria or tetanus antitoxin (Table III). Two of this group showed four-plus skin and positive conjunctival reactions to horse serum. Two of the 28 who never had received any serum yielded similar reactions. If all degrees of positive reactions to horse serum are included, then 7, or 58.3 per

*Tests were performed with 0.001 and 0.01 concentrations of dander extract (milligrams of nitrogen per cubic centimeter). In case of four-plus reaction to the former, higher concentration was not tested.

cent, of the 12 cases who previously had received serum, reacted to horse serum, and 18, or 64.5 per cent, of those who had not had any serum previously reacted to it. It appears, therefore, that there is very little difference in reactivity in these two groups. Since the series is small, no conclusions are attempted.

TABLE II

FORTY ALLERGICS TESTED WITH FOUR MAMMALIAN SERA: HORSE, GOAT, RABBIT, SHEEP

	HORSE SERUM					GOAT SERUM					RABBIT SERUM					SHEEP SERUM				
	REACTIONS																			
	4+	3+	2+	1+	0	4+	3+	2+	1+	0	4+	3+	2+	1+	0	4+	3+	2+	1+	0
	Intracutaneous Tests Using 1:10 Dilutions																			
Number of cases	4	2	6	13	15	4	0	3	14	19	3	0	6	12	19	4	0	8	10	18
	Conjunctival Tests Using 1:10 Dilutions																			
Number of cases	4	0	0	0	36	3	0	0	0	37	3	0	0	0	37	3	0	0	0	37

TABLE III

COMPARATIVE INCIDENCE OF POSITIVE SKIN AND CONJUNCTIVAL TESTS IN ALLERGICS WHO HAD PREVIOUSLY RECEIVED HORSE SERUM INJECTIONS WITH THOSE WHO HAD NOT

	NUMBER OF CASES	REACTIONS				
		4+ SKIN AND POSITIVE CONJUNCTIVAL	3+ SKIN AND NEGATIVE CONJUNCTIVAL	2+ SKIN AND NEGATIVE CONJUNCTIVAL	1+ SKIN AND NEGATIVE CONJUNCTIVAL	NEGATIVE SKIN AND CONJUNCTIVAL
Those who previously had horse serum injections	12	2	2	1	2	5
Those who never had any serum injections	28	2	0	4	12	10

SUMMARY

1. Four-plus skin and positive conjunctival tests were considered in this study as indicating allergy to serum. On the basis of the 40 allergics tested, 5, or 12.5 per cent, exhibited positive reactions to one or more sera; 4, or 10 per cent, to two or more sera; and 3, or 7.5 per cent, to all 4 sera. If all degrees of reaction are included, then 25 were completely negative to horse serum; 19 to goat and rabbit sera, and 18 to sheep serum.

2. Every case with a four-plus skin reaction to a serum also exhibited a positive conjunctival test, and vice versa. At no time was a positive conjunctival test obtained where the intracutaneous test to the serum was less than four plus.

3. a. Correlation between positive reactions to serum, and the corresponding dander antigen:

(1) Of the 3 cases that reacted to the 4 sera, one case also reacted to horse dander; one to goat and rabbit dander, and one to horse and rabbit.

(2) The case that reacted to goat and sheep serum evidenced no positive skin reactions to any danders.

(3) The case that reacted to horse serum alone also was positive to the corresponding dander.

b. correlation between positive reactions to animal danders, and the corresponding sera:

(1) Of 4 cases that reacted with a four-plus reaction to horse dander, all 4 also were positive by skin and conjunctival tests to the homologous serum.

(2) Of 2 cases that evidenced four-plus reaction to goat dander, one reacted to goat serum by skin and eye tests.

(3) Both cases that reacted with a four-plus reaction to rabbit dander also showed positive skin and eye tests to the serum.

(4) None of the 4 cases that reacted to sheep dander reacted to the serum. This may indicate that reaction to sheep dander is not very specific, or that there is a relatively low degree of association of sheep dander and sheep serum allergy.

(5) Twenty-eight, or 70 per cent, of the cases had never received any serum injections, while 12, or 30 per cent, did. There was very little difference noted in the number of positive reactions to horse serum in the two groups. Moreover, there were just as many positive reactions to goat, rabbit, and sheep sera where the question of prior serum injection was not a factor.

CONCLUSIONS

1. Atopic persons may be sensitive to the sera of one or more species of animals.

2. A four-plus skin and conjunctival reaction to a serum should be considered prima facie evidence that sensitivity to it exists, and its avoidance or the extreme care in its use is indicated.

3. Sensitivity to an animal dander is coincident in many cases with a sensitivity to the corresponding serum.

4. In our series we found very little difference in the frequency of positive reactions to horse serum in allergic persons who previously had had serum injected (diphtheria and tetanus antitoxin and diphtheria toxin-antitoxin), and in those who had never received any serum. Extreme care should be exercised in the use of therapeutic sera in any allergic patient.

5. In all cases the use of immune sera obtained from any species of animal is not devoid of the possibility of serum reactions. In all circumstances skin and conjunctival tests should be performed before a serum from any animal is injected. The presence of a conjunctival reaction is a danger signal which should not be ignored.

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CLINICAL CHEMISTRY

PROTHROMBIN STUDIES USING RUSSELL VIPER VENOM*

II. Relation of Clotting Time to Prothrombin Concentration in Human Plasma

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THE use of Russell viper venom instead of tissue extract as the source of thromboplastic substance in determining the prothrombin clotting time was reported first by Fullerton,[1] and later by Page and Russell.[2]

The end point of the test is taken as the number of seconds required for a fibrin web to form when calcium is added to a mixture of oxalated plasma and Russell viper venom. The time (result in seconds) obtained is generally considered to be inversely proportional to the concentration of prothrombin in the plasma. In order to determine the relation of prothrombin concentration to clotting time, using Russell viper venom as the thromboplastic agent, the study outlined here was carried out.

METHOD OF STUDY

Blood of normal persons and of patients attending an outpatient clinic is obtained. Only blood from patients not suspected of having liver damage or a vitamin K deficiency is used. Four and one-half cubic centimeters of venous blood are drawn into a dry syringe and mixed with 10 mg. of potassium oxalate in a centrifuge tube. The blood is centrifuged at 1,500 r.p.m. for five minutes and the oxalated plasma is drawn off. The test is performed as follows: 0.2 c.c. of oxalated plasma is pipetted into a small test tube (75 by 10 mm.) and 0.2 c.c. of Russell viper venom,† 1:10,000 solution, is added. Calcium chloride solution (1.11 Gm. calcium chloride per 100 c.c.), 0.2 c.c., is then added, and the stop watch is started. The tube is agitated for ten to fifteen seconds in a water bath (37.5° C.), then removed and tilted until separate discrete fibrin particles can be seen. The first appearance of the fibrin particles is taken as the end point. The prothrombin clotting time is determined on the undiluted plasma (100 per cent). Then a sample of the 100 per cent plasma is diluted with physiologic saline solution so that it represents 80 per cent plasma, and its prothrombin clotting time is determined. Serial dilutions are made containing,

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†Supplied as 'Stypven' Russell viper venom by Burroughs Wellcome & Co., Inc., New York, N. Y.

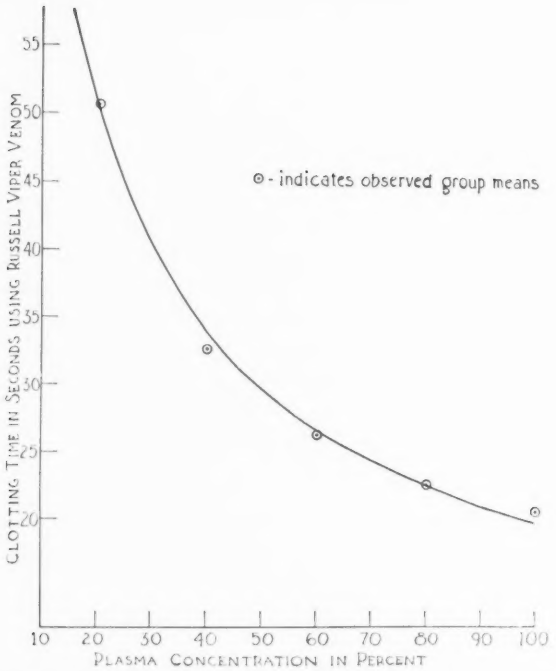

Fig. 1.—Showing the agreement between the plotted points and the curve calculated from the equation $T = 287.3X^{-0.581}$, where T is the clotting time in seconds and X is the percentage concentration of the plasma. Each plotted point is an average of 26 values.

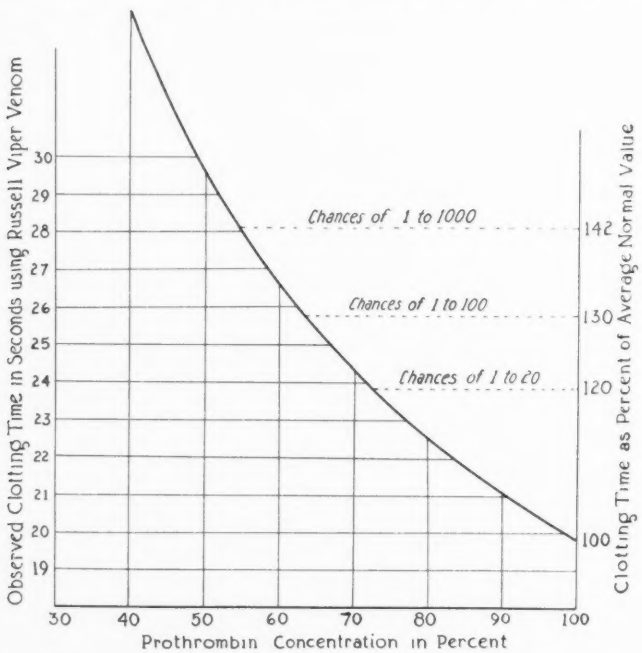

Fig. 2.—A chart giving the probability that an observed prothrombin clotting time or prothrombin concentration is normal. Case I. When the clotting time has been determined by the method described in this paper, or by some type of Quick's method giving essentially the same results (Page and Russell[2]), use the left-hand scale and then locate the corresponding point horizontally opposite on the curve. The nearest probability may then be read directly. Case II. When the average normal value has been found by experience to be appreciably different from twenty seconds, express the observed clotting time as a per cent of the average normal value and use the righthand scale. For example, if the average normal value in the experience of the laboratory has been found to be 22.2, and the particular observed value to be 30 (a figure which is about 135 per cent of the normal), the right-hand scale shows that the chances of this value being normal are quite small, i.e., between 1:100 and 1:1,000.

respectively, 60, 40, and 20 per cent of plasma, and the prothrombin clotting time is determined for each dilution. Each test is performed in triplicate, and the average time is taken for the result.

If the oxalated blood is allowed to stand for more than two hours before centrifugation, the prothrombin clotting time is usually increased, but occasionally it is decreased, the latter probably due to hemolysis with release of lecithin. If, on the other hand, the oxalated blood is centrifuged immediately, or at least within an hour, and the separated plasma is allowed to stand at room temperature for one and one-half to five hours, the prothrombin clotting time is usually increased in both the 100 per cent and 20 per cent plasma dilutions. These changes in prothrombin clotting time incident to standing led us to perform the test as soon as possible after the withdrawal of the blood.

RESULTS OF STUDY

The routine was carried out on 26 persons, and the resulting data are summarized in Table I. By extensive statistical treatment, the hyperbola of Fig. 1 was found to fit these data. Quick[3, 4] reports similar findings for data obtained by his methods.

TABLE I

PROTHROMBIN CLOTTING TIME DATA FOR DIFFERENT CONCENTRATIONS OF PLASMA USING SAMPLES OF BLOOD FROM 26 PATIENTS

	PERCENTAGE				
	100	80	60	40	20
Mean	20.76 sec.	22.81	26.60	33.10	51.36
Maximum	25.0	27.8	33.4	42.2	69.5
Minimum	15.4	16.5	17.0	20.0	40.4
Standard deviation	2.32	2.99	3.80	5.82	8.67

DISCUSSION

What is normal prothrombin clotting time? The question as to whether a particular clotting time is normal or abnormal is of great practical importance, and because of individual variations is difficult to answer. However, by means of Fig. 2* it is possible to estimate what the chances may be that a given high clotting time represents a high normal figure. If the chances are very slight that the high figure is normal, one may then be more certain that the high value is due to abnormal causes. Thus for example, if the clotting time of a patient's plasma is twenty-four seconds (corresponding to a plasma prothrombin concentration of about 70 per cent) should the value be considered a high normal, or does it indicate an abnormal clotting time? Reference to Fig. 2 reveals that the chances of such a clotting time being normal are only 1 to 20, and that the value must be considered on the border line. If the clotting time is twenty-six seconds corresponding to a plasma prothrombin concentration of about 63 per cent, the figure may quite safely be considered abnormal, since the chances are

*Fig. 2 was constructed as follows: All clotting times were expressed as logarithms, since the distribution then appears to be more nearly normal, and the expected mean and the standard deviation were found to be 1.2961 and 0.04955, respectively. Three multiples of the standard deviation (0.0815, 0.1153, and 0.1531) corresponding to the probabilities 0.05, 0.01, and 0.001 were added to the mean. The antilogs of these three sums were found, and the resulting clotting times were marked off on the curve of Fig. 2. The three factors for multiplying the standard deviation may be computed from such tables as Pearl's[5] areas of the normal probability curve or the probit tables of Bliss.[6]

1 to 100 against a normal clotting time being so high. If the clotting time is twenty-eight seconds, the plasma prothrombin concentration is only about 55 per cent, and the certainty that an abnormal condition exists is even greater, since the chances that it might be normal have now dropped to 1:1,000.

It is recognized that the same method of prothrombin determination in the hands of different workers does not always yield identical results. Since insufficient evidence exists as to how close an agreement may be expected between different laboratories, Fig. 2 has been made sufficiently adaptable to take care of a variety of conditions. When the average clotting time differs appreciably from the value reported in this paper, an adjustment may readily be made by expressing the clotting time as per cent of the mean.

Differences in the standard deviation which measures the variability about the mean must also be considered. Table I shows that the unselected data of this study are quite closely grouped about a mean value of about twenty seconds. If the data in question are much more scattered than these, the deviation of any suspected observation from the average normal value must be relatively greater before it can be considered abnormal. To take an extreme case, suppose that the standard deviation is twice as great as that reported in this paper. Then the three clotting times corresponding to chances of 1:20, 1:100, and 1:1,000, would have increased only to twenty-nine, thirty-four, and forty seconds, respectively. If, on the other hand, the standard deviation is less, then smaller deviations of the clotting time from normal would be significant.

SUMMARY

Plasma prothrombin dilution studies, using a modified Quick's method employing Russell viper venom instead of brain substance extract, have been carried out on 26 human beings who were found to be normal by clinical tests.

In our hands, using normal undiluted (100 per cent) human plasma, this test gave an average prothrombin clotting time of 20.6 seconds.

When the normal plasma was diluted with physiologic saline according to the following proportions, the prothrombin clotting time increased as the dilution was increased.

OXALATED PLASMA PER CENT	MEAN PROTHROMBIN CLOTTING TIME SECONDS
100	20.76
80	22.81
60	26.60
40	33.10
20	51.36

Graphs for computing the plasma prothrombin concentration (in terms of percentage of normal) from a known clotting time were worked out and are presented in Figs. 1 and 2.

The probability of an increased prothrombin clotting time being normal was given consideration. If the clotting time increases, the odds that it could still be normal decrease, and a statistical computation of this part of the study has been incorporated into a graph that may be used in connection with any method for determining prothrombin clotting time.

We wish to express our appreciation for the assistance and cooperation of Dr. Louis Bauman in whose laboratory this work was carried out.

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THE SIGNIFICANCE OF THE XANTHOPROTEIN REACTION IN RENAL INSUFFICIENCY AND OTHER CLINICAL CONDITIONS*

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In 1939 Kenny and Hubbard[1] reviewed the literature and reported their results based on 46 xanthoprotein determinations performed on 39 patients with renal insufficiency. Among other statements, they concluded that "aside from acute nephritis, the xanthoprotein reaction offers the best index of uremic intoxication and that uremic symptoms in individual cases may not parallel retention of urea nitrogen but rather that of aromatic substances."

The differential diagnosis between uremia and other clinical conditions simulating the uremic state is not always easy, especially so when a patient is seen for the first time in coma or in a comatose state. This is particularly true at the Cook County Hospital where numerous patients in whom early diagnosis is imperative, are brought into the emergency room. Although the blood nonprotein nitrogen and creatinine levels are routinely determined on all hospitalized patients, these are time-consuming procedures and not infrequently, therefore, accurate diagnosis must await these laboratory reports.

Impressed by this report and an earlier one by Steen[2] in which he analyzed 210 tests on blood and body fluids from living patients and from autopsies, we have studied the xanthoprotein reaction in renal insufficiency (uremia) and other clinical conditions in order to determine its efficacy in diagnosis and prognosis in uremia.

In 1924 Becher and his associates[3] reported an increase of aromatic substances—an ether-soluble group comprising phenols, cresols, aromatic oxyacids and indoxyl, and a nonether soluble group containing the amino acids, tyrosine, phenylalanine, and tryptophane—in the blood of patients with renal insufficiency. In 1925 Becher and Koch[4] reported markedly increased xanthoprotein values (80 to 250) in patients with uremia and indicated that uremia was less dependent on retention of nitrogenous products than on that of aromatic sub-

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stances, and that phenol poisoning resembled true uremia. Irdelp, Guchan, and Kazin[5] placed the normal xanthoprotein value between 15 and 35 and showed increased values in all types of renal insufficiency.

In our study we have performed 354 xanthoprotein determinations on 295 patients with various clinical conditions, according to the following technique, which is slightly modified from that originally described by Becher:[6]

To 3 or 4 c.c. of oxalated blood or serum, add an equal volume of 20 per cent trichloracetic acid and mix thoroughly. Allow to stand for several minutes and filter through a Whatman No. 40 quantitative filter paper. The filtrate must be absolutely clear, since a slight turbidity will obviate the result. If the filtrate is turbid, refilter it using a little pumice or talcum to clarify. To 1 c.c. of the clear filtrate add 1 drop of concentrated sulfuric acid, 0.5 c.c. of concentrated nitric acid, and a glass bead, and boil over a free flame for about a minute. Cool and add drop by drop about 1 c.c. of saturated sodium hydroxide till the mixture is alkaline. A marked golden color is obtained when phenolic bodies are present. A pale straw color is considered a negative test.

Quantitative Expression.—Make all measurements accurately and boil as evenly as possible so as to avoid excessive evaporation. Compare the color of the final mixture with a standard 0.3874 per cent solution of potassium dichromate. Prepare a series of standards as shown in Table I.

TABLE I

NO.	$K_2Cr_2O_7$ 0.3874%	WATER	DILUTION	XANTHOPROTEIN NUMBER
I	0.1 c.c.	0.9 c.c.	1:10	100
II	0.1 c.c.	1.9 c.c.	1:20	50
III	0.1 c.c.	2.9 c.c.	1:30	33⅓
IV	0.1 c.c.	3.9 c.c.	1:40	25
V	0.1 c.c.	4.9 c.c.	1:50	20

Compare the unknown in the tube macroscopically with the diluted standards or in a colorimeter. Thus, if the unknown solution matches standard No. II, the xanthoprotein number is 50; the colorimetric readings would be

$$\frac{\text{Standard set at 10}}{\text{Unknown reads 19.5}} \times 0.1 \times 1{,}000 = 50+.$$

Since normal equals 15-35, macroscopic comparison was considered sufficiently accurate.

RESULTS

Renal Insufficiency (Uremia).—Sixty-four xanthoprotein determinations were performed on 36 patients with uremia. Of these, 34 showed a rise in xanthoprotein values above 60, associated with a uremic rise in nitrogenous retention (i.e., 90 mg. nonprotein nitrogen per 100 c.c. of blood or higher), although the rise in the xanthoprotein reaction did not always correspond in degree to the rise in the blood nonprotein nitrogen level.

Xanthoprotein values ranged from 62 to 460, and the nitrogenous retention from 37 to 250 mg. per 100 c.c. In two cases the xanthoprotein values were below 60, while the blood nitrogenous level was above 90, i.e., of uremic

nature. Conversely, three cases showed marked xanthoprotein elevations (125, 167, and 70, respectively), while the nonprotein nitrogen level was only 60, 64, and 37, respectively. In only two of the 36 cases, therefore, the nonprotein nitrogen determination was of greater significance in diagnosing uremia than was the xanthoprotein reaction, whereas in three cases the reverse was true.

All but two of these patients died in uremia. In one patient repeated tests indicated a decreasing xanthoprotein value, although the nonprotein nitrogen remained elevated, and he was subsequently discharged; the other patient had elevated xanthoprotein and nonprotein nitrogen values, but left the hospital before repeat studies could be performed. Of the 34 patients who died, two had xanthoprotein values below 50. In one patient in whom 12 xanthoprotein determinations were performed over a period of several months, the highest value was 50, although nonprotein nitrogen values ranged from 93 to 212 mg. per 100 c.c.; in the second patient the xanthoprotein value was 35, and the nonprotein nitrogen was 239.

Six patients with no clinical evidence of uremia showed xanthoprotein reactions over 60: one patient each with nephrosclerosis, nephrosis, rheumatic heart disease, lysol poisoning, diabetic gangrene, and chronic nephritis. All were discharged. The patient with lysol poisoning presented a xanthoprotein value of 325, and a nonprotein nitrogen value of 41. He was discharged before repeat studies were made. By way of contrast another patient with lysol poisoning was admitted, but his xanthoprotein reaction was only 42. The difference of these values in two similar conditions may, we believe, be explained by the differences in time at which blood was secured for this study. Thus, the first patient was hospitalized within several hours of attempted suicide, and blood was obtained shortly thereafter at a time when the blood serum contained considerable amounts of phenol bodies; whereas in the second patient blood was not obtained until several days after hospitalization, at a time when the patient was convalescing and his blood serum apparently was relatively free of phenol bodies.

Cerebral Conditions.—Twenty-two xanthoprotein determinations were performed on 21 patients with various cerebral conditions, including seven patients with hypertensive encephalopathy and 10 with cerebral vascular accidents. In the seven patients with hypertensive encephalopathy the nonprotein nitrogen was elevated, at times markedly so, but in none was the xanthoprotein reaction persistently or markedly increased. In only one patient did the xanthoprotein value reach 50, but on repeating the test it promptly fell to 20. In the 10 patients with cerebral vascular accidents the xanthoprotein reaction was low, despite the fact that the nonprotein nitrogen was elevated in all and markedly elevated in three.

Nephritides.—Excluding the patients with uremia, 30 xanthoprotein reactions were studied on 22 patients with acute and chronic renal pathology. In three patients the xanthoprotein value was elevated beyond 60, but these left the hospital before repeated studies could be done. In the remainder the xanthoprotein reaction was not significantly elevated, although nonprotein nitrogen

levels were very high in many instances. None of the nine patients with acute nephritis showed significantly increased xanthoprotein values, the highest being 31.

Nine xanthoprotein studies were carried out on seven patients with genitourinary conditions, excluding nephritis. In none was the xanthoprotein value significantly elevated, although in two patients with prostate pathology and urinary retention, the nonprotein nitrogen was markedly increased and impending uremia was suspected.

Cardiac Conditions.—Ninety-six xanthoprotein reactions were studied in 81 patients with cardiac disease complicated by cardiac failure. In only one patient was the reaction higher than 60. In two it was over 50, and in five it reached 50. The remainder were below 50. Six patients had xanthoprotein values of zero; in two of these patients the nonprotein nitrogen was markedly elevated, being 125 and 154, respectively.

Gastrointestinal Conditions.—Forty-six xanthoprotein reactions were performed on 43 patients with various gastrointestinal conditions, including 20 patients with carcinoma and marked emaciation and 13 with peptic ulcers, most of which demonstrated active bleeding. In no instance was the xanthoprotein reaction elevated beyond 50, the great majority of reactions being below 20. In 10 the nonprotein nitrogen was markedly elevated.

Pulmonary Conditions.—Fifty-two determinations were performed on 52 patients, mainly with pulmonary tuberculosis or pneumonia, and nothing significant was revealed. The highest value was 41, most values being 20 or lower. Many of these patients had moderately increased nonprotein nitrogen levels and several showed marked increases.

Miscellaneous Conditions.—Thirty-five xanthoprotein reactions were determined on 33 patients with various clinical conditions (alkalosis, alcoholism, arthritis, avitaminosis, pernicious anemia, etc.) not previously included. With exception of the patients with lysol poisoning and diabetic gangrene previously referred to, none showed significant xanthoprotein elevations, although in seven the nonprotein nitrogen was markedly increased.

Included are three patients with coma of undetermined origin. In none was the xanthoprotein or nonprotein nitrogen significantly increased. Five patients had diabetes mellitus, and nothing of significance could be determined from the xanthoprotein reactions.

SUMMARY AND CONCLUSIONS

1. Although the range of normal values of the xanthoprotein reaction is accepted as 15 to 35, our study indicates that a more correct normal range is 0 to 50. Xanthoprotein values over 60 should be considered abnormal in all conditions.

2. Our data indicate that the only specific condition with significantly increased xanthoprotein values is uremia, although lysol poisoning must be kept in mind as a possibility.

3. As an indicator of impending or true uremia, the xanthoprotein reaction seems to be as accurate as the level of nitrogenous retention in the blood. Thus, in our series the xanthoprotein reaction was an accurate indicator in 94.4 per

cent of cases, whereas the blood nonprotein nitrogen level was significantly elevated in 91.6 per cent. A low xanthoprotein reaction does not always rule out uremia (5.5 per cent of uremia cases) nor does a high value invariably mean uremia.

4. The xanthoprotein value does not in all instances prognosticate the course of the patient with uremia, although with one exception all those with uremia whose xanthoprotein reactions were 100 or over died. Conversely, low xanthoprotein values do not preclude a fatal termination of uremia.

5. With the exception of those cases of uremia in which both the xanthoprotein and blood nonprotein nitrogen were definitely elevated, there was no consistent correlation between the xanthoprotein reaction and the level of blood nitrogenous retention.

6. The xanthoprotein reaction is valuable in differentiating cerebral conditions simulating uremia (pseudo-uremia) from true uremia. This is particularly evident in studying patients with hypertensive encephalopathy in whom no instances of elevated xanthoprotein values were noted despite moderate elevation of nonprotein nitrogen in many instances.

7. A low xanthoprotein value is significant in cases of extrarenal azotemia as occurs in dehydration states, following hemorrhage into the gastrointestinal tract, etc.

8. Although the xanthoprotein reaction has some limitations, it is an excellent supplementary test in the study of uremia, suspect uremia, pseudo-uremia, coma, and nephritis. It consumes little time, is simple to perform, and is inexpensive.

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THE EFFECT OF ANAPHYLACTIC SHOCK UPON BLOOD POTASSIUM AND CONCENTRATION*

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CONFLICTING reports relative to the results obtained following employment of potassium salts in allergic conditions[1-4] and the apparent lack of an adequate rationale for such a procedure on the basis of experimental blood potassium findings[5-7] led us to further investigate the subject from an experimental standpoint.

The present experiments were designed to correlate the blood picture with the symptoms manifested in the animals rather than with the sensitizing dose, time intervals, or shock dose. This will be made clear in the presentation of our results, which show the effects of rapidly and slowly fatal anaphylactic shock on potassium, relative red blood cell volume, and plasma specific gravity.

MATERIALS AND METHODS

Guinea pigs, weighing from 250 to 400 Gm., were used in this work. Blood obtained directly from the heart at various time intervals was drawn into a 2 c.c. tuberculin syringe, which contained a small amount of dry heparin (Connaught Laboratories, Toronto). Some of the heparinized blood was run immediately into a heavy wall 1 mm. capillary tube, 10 cm. long, which served as a hematocrit tube. Both the syringe and the tube were closed at both ends with broad rubber bands and centrifuged for fifteen minutes at 3,000 r.p.m. The hematocrit (relative red blood cell volume) was then read off directly in per cent by placing it on a millimeter scale.

The supernatant plasma in the syringe was used for both potassium and plasma specific gravity determinations. Potassium was determined in duplicate on 0.2 c.c. by the method of Truszkowski and Zwemer.[8] Plasma specific gravity was measured by the technique of Barbour and Hamilton,[9] and the proteins were calculated by the formula of Weech and co-workers.[10]

EXPERIMENTAL

Except for two groups of the controls, all animals were sensitized with 0.25 c.c. of horse serum. A second dose of horse serum was administered one to four weeks later by the intraperitoneal route. The "shock" animals fell into three clinical groups: (a) those that showed no evidence or only questionable evidence of shock (Table I); (b) those that died of anaphylactic shock after a period of

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TABLE I

ANIMALS RECEIVING TWO DOSES OF HORSE SERUM BUT SHOWING LITTLE OR NO CLINICAL EVIDENCE OF SHOCK

(Average time interval between initial and final values is one hour.)

GUINEA PIG NO.	1	2	5	7	32
Body weight	254	204	266	317	307
Shock dose of horse serum per 100 Gm. of weight	0.78 c.c.	0.9 c.c.	0.9 c.c.	0.6 c.c.	0.1 c.c.
Sensitization interval	1 wk.	1 wk.	2 wk.	2 wk.	3 wk.
Potassium (mg. per 100 c.c.)					
Values					
Initial	35.4	25.6	27.0	28.2	28.0
Intermediate	28.5	32.8	26.7	33.8	29.2
Final	26.5	28.8	28.8	32.8	26.7
Hematocrit (per cent)					
Initial	37.1	36.0	46.4	41.5	44.3
Intermediate	31.1	24.0	38.5	37.3	38.0
Final	37.0	26.4	31.1	33.3	35.5
Protein (Gm. per 100 c.c.)					
Initial	4.39	5.41	5.03	4.39	–
Intermediate	3.88	4.93	4.05	4.32	–
Final	4.52	4.73	3.37	3.74	–

TABLE II

ANIMALS RECEIVING TWO DOSES OF HORSE SERUM AND SHOWING SHOCK OF SLOW ONSET AND MODERATE DEGREE

(Average time interval between initial and final values is four hours. Complete findings on animal No. 10 are shown in Graph 4.)

GUINEA PIG NO.	9	10	13	39	40	41
Body weight	305	285	319	270	344	431
Shock dose of horse serum per 100 Gm. of weight	0.8 c.c.	0.8 c.c.	1.2 c.c.	1.5 c.c.	1.5 c.c.	1.5 c.c.
Sensitization interval	2 wk.	2 wk.	3 wk.	3 wk.	3 wk.	3 wk.
Potassium (mg. per 100 c.c.)						
Values						
Initial	27.9	26.4	25.7	30.7	33.1	22.2
Intermediate	29.9	29.9	29.2	37.0	29.5	18.8
Final	39.6	46.5	43.0	38.8	37.0	27.1
Hematocrit (per cent)						
Initial	46.0	45.2	43.7	35.0	45.8	44.1
Intermediate	50.0	25.9	50.0	25.9	34.7	42.9
Final	38.9	38.8	46.7	30.5	32.3	49.0
Protein (Gm. per 100 c.c.)						
Initial	4.49	5.51	5.06	4.56	5.85	5.85
Intermediate	3.57	4.59	4.22	4.42	4.93	5.12
Final	3.78	4.42	4.04	4.69	5.17	5.27

TABLE III

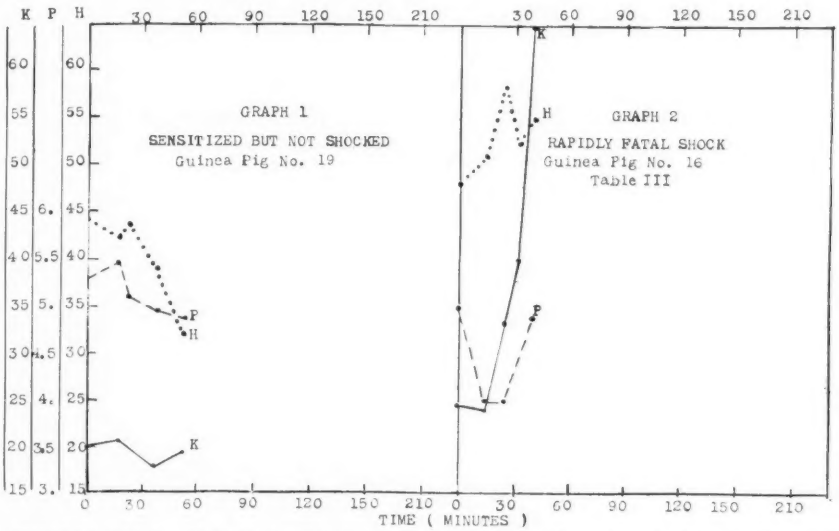
ANIMALS RECEIVING TWO DOSES OF HORSE SERUM AND SHOWING SHOCK OF RAPID ONSET AND SEVERE DEGREE*

(Average time interval between initial and final values is forty-five minutes. Complete findings of animal No. 16 are shown in Graph 2.)

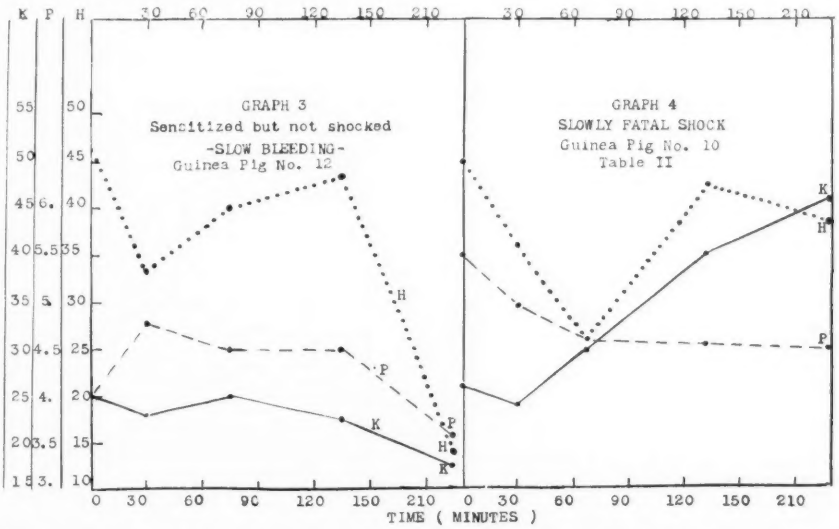
GUINEA PIG NO.	6	8	14	16	17	25	28	27	29	33
Body weight	267	222	300	368	231	304	276	283	349	320
Shock dose of horse serum per 100 Gm. of weight	0.75 c.c.	0.9 c.c.	2.2 c.c.	2.7 c.c.	3.1 c.c.	1.5 c.c.	1.5 c.c.	1.5 c.c.	2.0 c.c.	2.0 c.c.
Sensitization interval	2 wk.	2 wk.	3 wk.	4 wk.	4 wk.	2 wk.	3 wk.	2 wk.	3 wk.	4 wk.
Potassium (mg. per 100 c.c.)										
Values										
Initial	31.7	28.0	30.4	24.5	24.1	31.3	27.0	31.5	31.3	20.3
Intermediate	Insuf.	Insuf.	37.5	33.5	29.7	31.4	31.0	34.2	35.4	34.5
Final	Insuf.	42.8	55.6	65.8	52.0	37.0	40.0	Insuf.	41.3	34.8
Hematocrit (per cent)										
Initial	38.4	40.5	44.0	48.1	49.1	47.6	43.8	44.6	43.7	49.6
Intermediate	Insuf.	28.5	44.7	58.6	46.7	41.6	42.1	38.9	47.5	62.7
Final	44.4	42.8	57.6	55.0	53.4	36.5	42.2	48.2	48.2	61.2
Protein (Gm. per 100 c.c.)										
Initial	4.46	4.93	4.42	5.27	5.14	4.90	4.63	3.88	4.69	4.97
Intermediate	Insuf.	4.63	4.97	4.02	5.14	4.42	4.49	4.42	4.22	4.52
Final	4.93	5.92	4.90	4.97	5.48	3.98	4.39	Insuf.	5.03	4.49

*Death occurred within one hour.

three or more hours (Table II, Graph 4); and (c) those that went into shock and were dead within an hour (Table III, Graph 2). The control series were of four types: (a) three animals sensitized but not shocked, and bled rapidly at intervals corresponding to those undergoing intense shock, one of which is shown



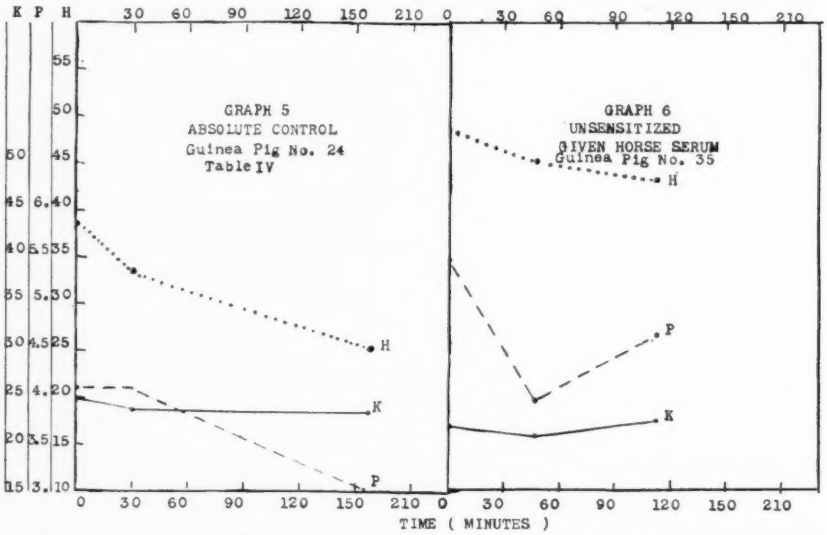
Graphs 1 and 2.—Note that potassium rise in shocked animal is out of proportion to hemoconcentration.
H = hematocrit (packed red blood cell volume per cent).
P = plasma proteins (grams per 100 c.c. as calculated from plasma specific gravity).
K = potassium (milligrams per 100 c.c. of plasma).



Graphs 3 and 4.—Note the steady rise in plasma potassium despite some hemodilution.

in Graph 1; (b) three bled at less frequent intervals which corresponded to slow shock (example in Graph 3); (c) three guinea pigs given large amounts of horse serum without previous sensitization (example in Graph 6); and (d) three controls which had received no injections of any kind and were used to check the effects of blood loss and accuracy of technique (Table IV, Graph 5).

Animals in which anaphylactic shock of rapid onset and marked intensity was produced had a pronounced elevation in plasma potassium which was greater than could be accounted for by the hemoconcentration as measured by red blood cell volume and plasma protein. This was in contrast to the control group which with equally rapid sampling showed hemodilution, lowered plasma protein and, in some cases, a lowered potassium. The changes were roughly proportional to the amount of blood lost by sampling; this blood loss is an important factor in increasing the severity of anaphylaxis in small animals.[11]



Graphs 5 and 6.—Note the constancy of plasma potassium despite hemodilution resulting from bleeding.

Examples from the significant groups are given in Graphs 1 to 6, and demonstrate quite clearly the effect of anaphylactic shock on the three blood constituents studied. If one compares Graphs 1 with 2, the difference between the shocked and unshocked animals, in so far as plasma potassium is concerned, is quite striking. The same may be said regarding Graphs 3 and 4, the sensitized unshocked animal showing essentially only those changes which may be attributed to blood loss, whereas the shocked animal shows a steadily rising plasma potassium level.

Also, if one compares all the graphs of animals in the control groups (Graphs 1, 3, 5, 6), the general similarity is apparent.

DISCUSSION

A rise in plasma potassium has been shown to be a feature of anaphylactic shock,[5-7, 12] of neurogenic and traumatic shock,[13, 14] and of acute allergic reactions.[15] Our results confirm the plasma potassium rise during the course of anaphylactic shock and show it to be definitely out of proportion to the degree of hemoconcentration and increased plasma density.

The findings in regard to changes in blood concentration are confirmatory of the work of Petersen and Levinson,[16] of Simonds,[17] and of Manwaring and

others,[18-21] most of whom ascribe these changes to disturbances of endothelial permeability occurring during the course of the anaphylactic reaction.

Black and Kemp[22] reported a consistent increase in plasma density during the course of anaphylactic shock in guinea pigs. Our findings do not agree, but the time factor, or the amount of blood taken, may explain this apparent contradiction. Other authors report a lack of constant variation in total protein during the course of anaphylactic shock, but note consistent shifts in the albumin-globulin ratio and in fibrin values.[23-26]

TABLE IV

ANIMALS RECEIVING NO HORSE SERUM

(Average time interval between initial and final values is two hours. Complete findings of animal No. 24 are shown in Graph 6.)

GUINEA PIG NO.	21	24	37
Body weight	269	233	425
Shock dose of horse serum per 100 Gm. of weight	0	0	0
Sensitization interval	0	0	0
Potassium (mg. per 100 c.c.)			
Values			
Initial	27.3	24.7	23.7
Intermediate	27.0	23.7	25.3
Final	28.4	23.1	26.6
Hematocrit (per cent)			
Initial	42.8	38.9	46.0
Intermediate	40.4	33.3	42.6
Final	38.3	25.4	40.2
Protein (Gm. per 100 c.c.)			
Initial	4.83	4.15	5.27
Intermediate	3.91	3.96	5.24
Final	3.78	2.96	4.69

The mechanism of the potassium rise still remains to be demonstrated. Since potassium is the principal intracellular base, as contrasted to sodium, which is the essential basic ion of plasma and interstitial fluid, it seems reasonable to assume that an increase of plasma potassium represents a loss of potassium by cells due to either a change in cell membrane permeability or a disruption in the hypothetical intracellular potassium colloid complex, or both.

Since it has been shown that injections of potassium salts are capable of producing many symptoms of shock,[6, 27] it seems probable that the increase in plasma potassium, occurring during the course of anaphylactic shock, accounts in some measure for the symptoms which are characteristic of this condition. In view of the close relationship between anaphylaxis and allergy, these findings suggest that the use of large amounts of potassium in allergic conditions is contraindicated rather than indicated.

SUMMARY

1. Thirty-four guinea pigs were used to determine the effect of anaphylactic shock upon plasma potassium and blood concentration in terms of plasma protein and red blood cell volume per cent.

2. A constant rise in plasma potassium was demonstrated, together with a fairly constant hemoconcentration in terms of red blood cell volume per cent. The potassium rise was relatively much greater than the degree of hemoconcentration and was roughly proportional in degree to the severity of shock. No constant change in plasma protein was demonstrable.

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THE CHEMISTRY OF INFECTIOUS DISEASES*

III. Polarographic Studies of the Behavior of Normal and Pneumococcus Infected Dog Sera Toward Denaturation Agents and Enzymes

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VERY little information is available on the low molecular weight protein derivatives of serum. Lack of convenient methods of handling proteoses, peptones, and polypeptides is responsible for their neglect in studies of protein metabolism.

The lower derivatives (peptones and polypeptides) are not precipitated by salting-out, and are soluble in trichloracetic and sulfosalicylic acids, but the addition of tungstic or phosphotungstic acid to a sulfosalicylic acid filtrate of serum will give a voluminous precipitate.

It is not known whether the serum protein derivatives of low molecular weight possess any physiologic activity of their own. Recent evidence indicates, however, that the peptone fraction† of blood serum may play a part in health and disease. It has been demonstrated recently[1] that during experimental type I pneumonia in dogs this peptone fraction greatly increases and remains abnormally high long after clinical evidences of infection have disappeared (fever, complete resolution of the congested areas of the lung as shown by x-ray, and the physical well-being of the animal). A positive polarographic cancer reaction according to Brdicka's technique[2] probably also depends upon the relative amounts of serum peptones.

The abnormal increase in the peptone fraction of the blood during pneumococcal infection may be only a reflection of a general increased metabolism, possibly associated with fever, or it may be concerned with the catabolism of the proteins at the site of infection. These two possibilities as to the probable source of the increased peptone fraction during infections are under investigation. However, there is another possible explanation. If one or more of the serum proteins should lose their normal stability under the stress imposed upon the organism by the invading microorganism, an abnormal tendency to disintegrate may occur. Certain facts suggest that this might actually take place, and this paper deals with the results of the investigation.

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†Peptone fraction will be defined as that part of blood serum which is responsible for the typical polarographic protein wave in $Co^{+++}.NH_4Cl$-buffer after precipitation of the proteins by sulfosalicylic acid.

EXPERIMENTAL

The stability of dog sera toward denaturation and enzymatic hydrolysis before and after experimental type I pneumococcus infection was determined polarographically by comparing the relative rates at which the polarographic wave heights of normal and of infected sera changed during denaturation. Preliminary experiments with urea, guanidine hydrochloride, alkali, and wetting agents showed that 8 M concentrations of the first two denaturing agents inhibited the formation of the typical polarographic wave to such an extent as to preclude their use in this study, while 1 N potassium hydroxide and Aerosol OT gave satisfactory results with the polarograph.

The dogs used in this study were normal, adult mongrels, weighing 34 to 48 pounds, which had lived in our kennel for at least five weeks prior to infection. Diet, care, the method of blood sampling, and the mode of infection with type I pneumococci have been described previously.[1] Denaturation of the sera was obtained by the addition of 10 c.c. of 1 N potassium hydroxide (or 10 c.c. 0.025 M Aerosol OT) to 4.0 c.c. of serum at room temperature. After various intervals, 1.4 c.c. of the mixture were added to 1 c.c. of 20 per cent sulfosalicylic acid and filtered after standing for five minutes. One-half cubic centimeter of the filtrate was electrolyzed in 5 c.c. of the previously described cobaltic ammonium chloride buffer[1] at 1/100 sensitivity of the galvanometer. The zero time of action was obtained by adding 2 c.c. of a 1:1 mixture of 1 N potassium hydroxide and 20 per cent sulfosalicylic acid directly to 0.4 c.c. of serum, filtering after five minutes' standing, and treating 0.5 c.c. of the filtrate as described above. The conditions of the enzymatic hydrolysis experiments were so chosen that just enough enzyme was present to insure a polarographically measurable digestion under optimum conditions of pH and temperature without having the waves produced by the enzymes themselves mask those produced by the partially digested proteins. The following procedure was finally adopted: 2 c.c. of 10 mg. per cent pepsin (Difco, spongy granular 1:3,000) in 1 N hydrochloric acid were added to 10 c.c. of either normal or infected serum. After a definite period of incubation at 39° to 40° C., 0.48 c.c. of the solution was withdrawn, added to 2 c.c. of 1:1 mixture of 1 N potassium hydroxide and 20 per cent sulfosalicylic acid. After standing for five minutes the solution was filtered. One-half cubic centimeter of the filtrate was analyzed polarographically in 5 c.c. of the cobaltic ammonium chloride buffer with the galvanometer sensitivity set at 1/100. For the tryptic digestion, 8 c.c. of serum were added to 2 c.c. of a 1 per cent trypsin (Difco, 1:110) solution in M/15 phosphate buffer at pH 8. Otherwise the procedure outlined for the peptic digestion was followed, except that 0.5 c.c. of the digestion mixture was added to 2 c.c. of the potassium-sulfosalicylate instead of 0.48 c.c.

All polarograms are self-recorded photographs of the galvanometer deflection. The increase in galvanometer deflection between –0.9 volts and the minimum of the double wave (–1.65 volts) determines the wave height. Any change from the normal is interpreted as being due to concentration changes in polarographic active groups of the peptone fraction of the serum after infection.

The rate at which the wave height increased, above that given by normal serum, by such criteria, would be indicative of an increased rate of denaturation of the serum proteins after infection.

RESULTS

Alkaline Denaturation.—Two typical sets of polarographic recordings of the peptone fractions from alkali-treated dog sera are reproduced in Fig. 1. Curve I, Fig. 1, represents the polarograms obtained from the filtrates of a normal dog serum, while curve II corresponds to that of the serum from the same dog after a mild attack of experimental pneumococcal pneumonia. The severity of infection in all cases is judged by fever, lung congestion as shown by x-ray, and by the general appearance of the animal. Upon the addition of

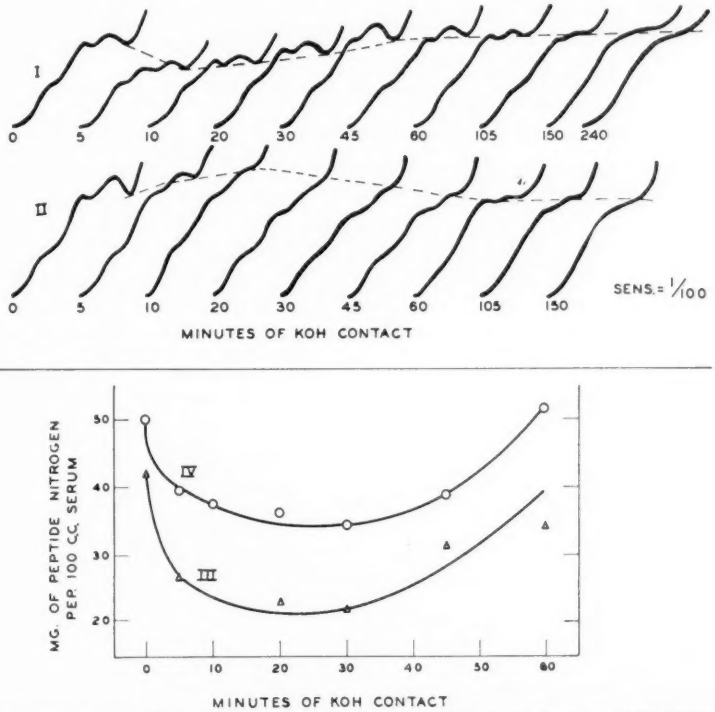

Fig. 1.—Polarograms and nitrogen values of the serum peptone fraction of alkalidenatured dog sera before (I and III) and after (II and IV) type I pneumococcus infection.

alkali to normal serum, a decrease in wave height occurs in the sulfosalicylic acid-soluble serum fraction during the first five to ten minutes of alkali content, followed by a rise until a fairly constant wave height is reached at sixty minutes (curve I). Further contact with alkali tends to destroy the double wave, a single wave appearing instead after the 150th to 240th minute. Denaturation of the serum from the same dog after infection (curve II) follows an entirely different course. A rise in wave height occurs during the first five to ten minutes of alkali contact, followed thereafter by a fall to near the original level of the untreated serum (zero time) which, incidentally, is also approximately that obtained from normal serum after sixty minutes' denaturation. The two polarographic denaturation curves are thus entirely different from each other.

Wherever the peptone fraction of normal serum shows a rise in wave height, the corresponding one from the infected serum decreases, and vice versa. There exists still a further difference in the polarograms of the two types of sera. The change from a double wave to a single wave occurs much sooner when the serum from an infected dog is denatured. Depending upon the severity of infection, this change in the structure of the polarograms has appeared as early as forty-five minutes after alkali addition, and always after sixty to one hundred and five minutes, while normal serum never showed the disappearance of the double wave prior to one hundred and fifty minutes of alkali contact.

If the assumption is correct that an alteration in the structure of the serum proteins occurs during infection and is responsible for the observed differences in wave heights during the early, and again in the late periods of alkali contact, then it should follow that the sudden rise in wave height during the first five to ten minutes after potassium hydroxide addition should be most pronounced in cases of severe infections, and less so during the early periods of pneumococcal invasion, when lung inflammation is still restricted. To test this, the spreading of the infection in the lungs was followed by x-ray, and blood samples were drawn at various stages of infection, the serum was denatured as described, and the wave heights of the peptone-polarograms were plotted against time of alkali content. In this manner a series of denaturation curves are obtained (Fig. 2) which fully substantiate the original assumption. The more severe the infection, the greater and more abrupt is the initial rise in wave height, while the polarograms of the normal serum are again entirely opposite. The zero time values in Fig. 2 express, in terms of polarographic wave heights, the preexisting concentrations of peptone material of the sera prior to denaturation and confirm our previous observations[1] that an increase in the serum-peptone fraction takes place during pneumonia. Interestingly enough, the blood sample drawn twenty-four hours after infection still exhibits a decrease in wave height during the first ten minutes after alkali addition, although it is not as pronounced as for normal serum, while its zero time value demonstrates that an accumulation of peptone material already has begun.

In polarography, changes in wave height merely indicate that concentration changes have taken place in the material which are responsible for the wave. Under our experimental conditions only cystine-containing proteins will give the wave. If the peptones which accumulate during pneumonia, and which are split off from the serum proteins by alkaline denaturation always possess the same average cystine composition, then the nitrogen content of the peptone fraction should fluctuate in a similar manner to that of the corresponding polarograms. Micro-Kjeldahl nitrogens were, therefore, run on some of the peptone fractions at various time intervals of alkaline denaturation, the peptone nitrogen being determined as the difference between the nitrogen value after sulfosalicylic acid and after tungstic acid precipitation of the serum. Curves III and IV in Fig. 1 are graphs of such analyses performed on the serum filtrates which correspond to the polarographic curves I and II of Fig. 1, respectively. It is evident from an inspection of curves II and IV that the peptone fraction of the infected serum (curve IV), as expected, contains more peptone nitrogen

throughout the entire denaturation range than the normal one. Entirely unexpected were the findings that the nitrogen of both the normal and the infected serum filtrates decreased during the first twenty minutes of alkali contact, and then increased thereafter until, after sixty minutes, approximately the initial values were obtained. The changes in peptone nitrogen of normal denatured serum thus ran roughly parallel to those in polarographic wave height. However, this relationship does not hold for those from infected serum. The possible significance of this discrepancy will be discussed later, but it should be pointed out that a direct dependency of wave height to peptone concentration can be readily demonstrated. If, for instance, the observed lower wave height of normal, undenatured serum (i.e., zero times in Figs. 1 and 2) is due to less peptone material than is found in an equal volume of untreated serum of the

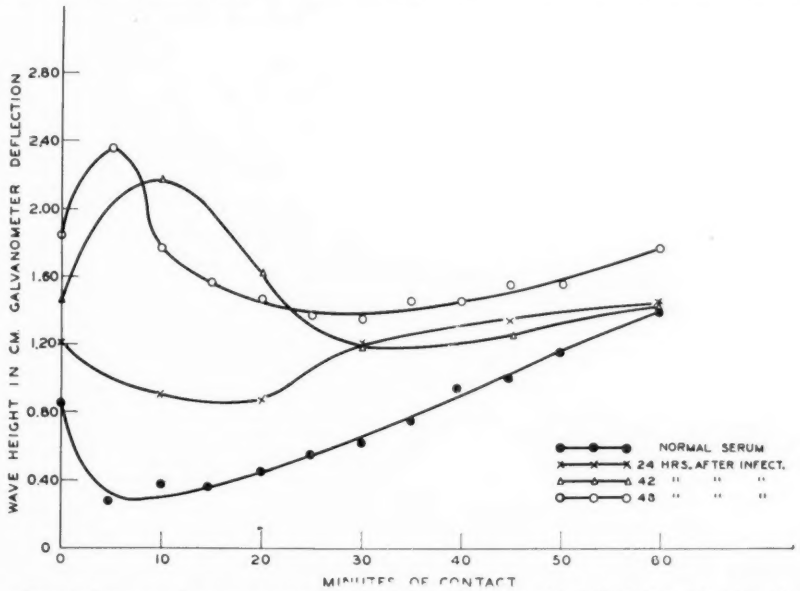

Fig. 2.—The influence of pneumococcus infection upon the characteristics of normal serum-peptone curves during alkaline denaturation.

same dog after infection, then it should be possible to match the waves obtained from the latter by merely increasing the amounts of the former. The results of such experiments are shown in Fig. 3. Each polarogram in the upper row was obtained by electrolyzing 0.5 c.c. of serum filtrate (equivalent to 0.083 c.c. of original serum), the blood of which was drawn at various stages of infection. The polarograms of the lower row were obtained from normal serum only, except that the serum concentrations were increased above 0.083 c.c. in such manner that each wave matched in height one of the current-voltage curves of the infected sera. It is evident that the polarograms due to 0.100 c.c., 0.133 c.c., 0.166 c.c., and 0.199 c.c. of normal serum are identical, within the error of the method, with those from 0.083 c.c. of sera drawn from the same dog, twenty-four, forty-two, forty-eight, and ninety hours after infection. Similarly, normal and infected peptone polarograms of denatured sera can be matched, either by increasing the concentration of the former or by decreasing that of the latter.

When different denatured sera are compared, the length of time during which the alkali acts must be the same, since alkali contact eventually causes all peptone polarograms, whether obtained from normal or from infected sera, to lose their double wave. The sera from which the curves in Fig. 4 were obtained were consequently denatured in each case for forty-five minutes with 1 N potassium hydroxide. The infected serum was drawn from the same animal during the

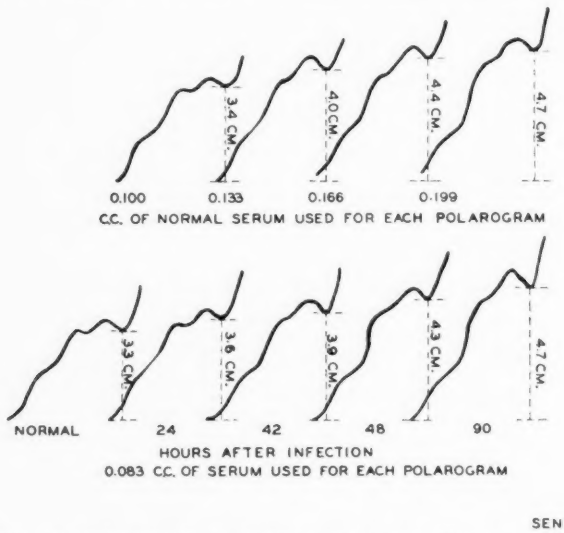

Fig. 3.—The effect of concentration of undenatured serum filtrates from normal and infected dogs upon the polarographic wave height.

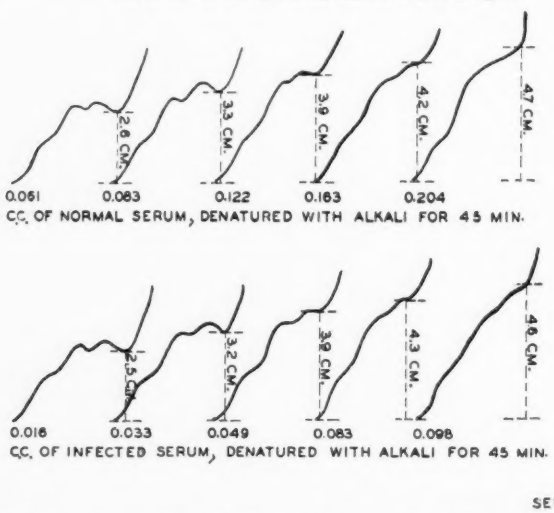

Fig. 4.—The effect of concentration of denatured serum filtrates from normal and infected dogs upon the polarographic wave height and wave structure.

course of an infection of medium severity. In order to match the polarograms from 0.061 c.c., 0.083 c.c., 0.122 c.c., 0.163 c.c., and 0.204 c.c. of normal, denatured serum, filtrates containing 0.016 c.c., 0.033 c.c., 0.049 c.c., 0.083 c.c., and 0.098 c.c. of infected serum, respectively, were required. The double wave begins to straighten out when the peptone fraction from 0.163 c.c. of normal or

0.083 c.c. of abnormal serum is electrolyzed, and it disappears completely, in favor of a single wave, when the concentrations of normal and infected serum reach 0.204 c.c. and 0.098 c.c., respectively. These experiments (Figs. 3 and 4) clearly show: (1) that wave height differences in the polarograms of the peptone fraction from dog sera, before and after pneumococcal infection, reflect concentration changes in the polarographically active principle, both for untreated as well as for denatured sera; (2) that, within the limits of concentrations studied here, no alteration in the structure of the polarographic double wave occurs with increasing concentrations of peptone material from untreated serum, but that alkali-denatured serum reaches a limit in concentration above which the double wave changes over to a single wave; (3) that the disappearance of the double wave occurs at considerably lower serum concentrations of infected serum than of normal serum.

Wetting Agents.—Alkaline denaturation at room temperature unquestionably produces more than one change in the structure of a protein molecule. Rupture of secondary linkages, partial hydrolysis of peptide bonds, and a breakdown of disulfides undoubtedly occur. Denaturation by wetting agents, such as Aerosol OT, Duponol PC, Duponol 80, and others[3] probably proceeds to a much more limited extent, possibly involving only the liberation of sulfhydryl groups from cystine. Since the polarographic protein wave is supposedly given by -SH-protein as well as by -S-S-protein groups, a preliminary change of the oxidized form to the reduced form by wetting agents should not increase the polarographic wave height, unless a liberation of previously unavailable thiol groups had occurred. If cyclic sulfur compounds of the thiazoline type are part of a protein molecule,[4] a liberation of new thiol groups by the action of alkali is conceivable and would at least partially account for the increase in polarographic active groups. No information as to the action of wetting agents on these heterocyclics is available. It is, however, unlikely that the wetting agents would liberate thiol groups from thiazolines. When the influence of Aerosol OT on serum was studied, it was found that the polarographic peptone wave, even after twenty-four hours of Aerosol OT contact, was identical with that of the same serum at zero time of Aerosol OT action. This was true for normal serum as well as for the serum of the same dog after a severe pneumococcal infection, although the relative heights of the two sera naturally differed, that from normal serum being the lower.

Enzymes.—The observed differences in the behavior of normal and of infected sera toward alkali, and the consequent belief that an alteration in the chemical structure of the proteins had occurred during infection, prompted us to investigate their enzymatic hydrolysis. It was hoped that pepsin and trypsin would digest normal serum proteins at a rate measurably different from that of infected serum; however, no differences were observed. Consequently, only the digestion polarograms of normal serum are shown in Fig. 5, where curve I represents the peptic digestion and curve II the tryptic one. The first current-voltage curve in each series is that of the blank, obtained by substituting saline for the serum. Its wave height must be subtracted from the serum-digestion polarograms if quantitative comparisons are to be made. Qualitatively, it is

quite evident that the polarographic technique lends itself well to proteolytic studies. Any increase in wave height above that of the zero time is due to proteolysis and is, therefore, a measure of the degree of enzymatic degradation that occurs. Furthermore, it permits an approximate estimation of those protein degradation products which were formed during digestion. These are not precipitable by sulfosalicylic acid but have a larger molecular weight than that of free cystine and cysteine, since these two amino acids in the free state give only a wave in cobaltous ammonium chloride buffer but not in the cobaltic buffer used here. Special attention should be called to the straightening out

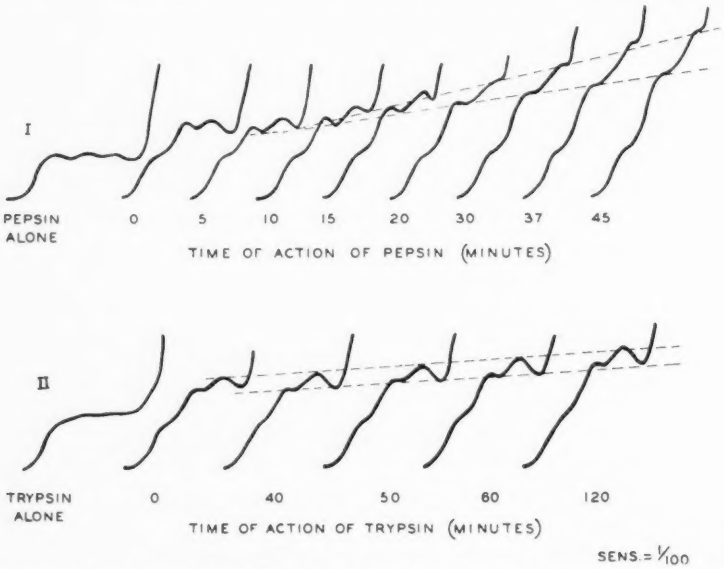

Fig. 5.—Polarograms of deproteinized dog sera at various stages of peptic (I) and tryptic (II) digestion at 40° C.

of the double wave during peptic digestion of serum, and of the failure to do so in case of tryptic digestion. A change in the structure of the polarographic protein double wave has so far been associated with denaturation and not with hydrolysis. Peptic activity seems to resemble denaturation in this respect, because the straightening out effect is also obtained by heat denaturation[5] or ultraviolet light irradiation[6] of serum albumin. Interestingly enough, ultraviolet irradiation of egg albumen causes a decrease in wave height[6] instead of the increase observed with serum albumin.

The relatively slower digestion of the serum proteins by trypsin than by pepsin is probably due to differences in the enzyme preparations. Since we were interested only in the relative rates of digestion of normal and of infected serum by the same enzyme preparation, no attempt was made to compare the different enzymes.

DISCUSSION

It is well known that when alkali is added to native proteins, denaturation occurs. This is followed by cleavage of the denatured protein into fragments, the sizes of which decrease as the action of the alkali proceeds. These degrada-

tion products are not very clearly defined, but have been classified as metaproteins, proteoses, peptones, polypeptides, and peptides, according to certain arbitrarily defined characteristics. Those products of a molecular weight smaller than that of metaproteins are not precipitated by sulfosalicylic acid; consequently the magnitudes of the polarographic wave heights, as shown in Figs. 1 and 2, are a measure of the smaller protein molecules only. With these facts in mind an attempt will be made to present a mechanism which will describe the course of the alkaline degradation of normal and of pneumococcus-infected dog sera.

If alkali were added to a serum which contained native proteins only and which was free of all other protein degradation products, the sulfosalicylic acid filtrates of such a serum would give no polarographic waves until the first molecules of proteoses were formed. There is an induction period, the length of which would be determined by the rate at which native proteins become hydrolyzed to the metaprotein state (k_1) and by the average of the rates of degradation of the latter to proteoses (k_2). On the other hand, if the higher proteins coexisted with proteoses and peptones in untreated serum, then the filtrate after the addition of sulfosalicylic acid would give a polarographic wave, the magnitude of which would reflect the concentration of pre-existing peptone molecules. The course of the reaction after the addition of alkali to such a serum would be determined by the relative amounts of pre-existing native proteins, metaproteins, and peptones, as well as by the relative rates of k_1, k_2, and k_3, the latter being the average of the rates of the destruction of the sulfosalicylic acid-soluble protein fractions to particles sufficiently small not to give a polarographic wave in trivalent cobalt buffer. We know that cystine has no influence on the wave in this medium and that glutathione gives a barely visible wave. Preliminary work, which we hope to report soon in detail, has shown that if definite values are assigned to the rate constants in such a manner that $k_1 > k_2 > k_3$, then graphs can be plotted which show the same characteristics in their shapes as the curves given in Figs. 1 and 2, both for normal and for infected sera. In the case of normal serum, which contains relatively small concentrations of peptones, a pronounced induction period would be expected during which no new proteoses enter the sulfosalicylic acid filtrates. However, during this induction period, some of the pre-existing peptones of very low molecular weight are changed to such an extent that they become polarographically inactive, causing a gradual lowering of the wave heights of the polarograms. This period is followed by an interval of peptone accumulation, since the rate of proteose formation from protems has been postulated as being more rapid than the destruction of peptones, and it is manifested by a rise in the wave height. After all the proteins have been degraded, the polarograms begin to decrease. Experiments carried out, but not included in Figs. 1 and 2, wherein the alkali was allowed to act on the serum proteins for a period of twenty-four hours, have shown that filtrates obtained after sulfosalicylic acid precipitation no longer produce the characteristic polarograms. Further evidence of the correctness of such a reaction mechanism is also borne out by our observation that the nitrogen values of the filtrates decrease during the first fifteen minutes of alkali contact, but then rise again to the original value or above it (Fig. 1, curve III).

The large accumulation of peptone material in the serum of infected dogs prior to the addition of alkali suggests that a degradation of native serum proteins had occurred in vivo. Under these circumstances it is reasonable to assume that the sulfosalicylic acid-insoluble fraction does not consist entirely of native proteins, but is a composite of native, denatured, and metaproteins. In such a case no induction period should be expected, as the experimental evidence confirms, and the formation of new proteoses would exceed the rate of peptone destruction, since it was postulated that $k_1 > k_2 > k_3$. Such a mechanism, therefore, accounts for the initial high level in polarographic wave height at zero time, the rapid initial rise after the addition of alkali, and a more sharply defined fall after all of the macroproteins have been converted to sulfosalicylic acid-soluble molecules. The initial fall in the nitrogen values of the peptone fraction of the infected serum, as shown in Fig. 1, curve IV, is the only datum not in complete accord with the reaction mechanism given above. Additional data are being accumulated which, it is hoped, will further clarify this point.

It is thus seen that the observed differences between the action of alkali on normal and on infected sera can be accounted for satisfactorily by the partial in vivo destruction of native serum proteins to molecules of lower molecular weight during experimental type I pneumococcus infection in dogs.

SUMMARY

The polarographic protein reaction has been used to study rates of denaturation of serum proteins. No appreciable differences have been found between rates of denaturation of serum from normal and from type I pneumococcus-infected dogs when wetting agents, or pepsin and trypsin are used.

Alkali produces changes in the polarographic rate of denaturation curves, which differ markedly for normal and infected sera. It is suggested that the partial in vivo destruction of the serum proteins during infection to molecules of the size of metaproteins, proteoses, and peptones can account satisfactorily for the observed differences.

We gratefully acknowledge the invaluable help and the unstinted cooperation we received in this study from Dr. W. Harry Feinstone of this laboratory. Dr. Feinstone, while actively studying related phases of pneumococcal infection in dogs, kindly took over the culturing of the pneumococci and the actual infection of the dogs, and followed the progress of the disease systematically with x-ray photographs.

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LABORATORY METHODS

GENERAL

THE INTRAVENOUS ADMINISTRATION OF OXYGEN*

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THE purpose of this paper is to present a new apparatus which is designed for the carefully controlled intravenous administration of oxygen.

The idea was conceived that anoxemia could be relieved by the intravenous administration of oxygen. It was believed that this method of administration could be made available for those cases in which oxygen therapy, by the usual route, was not adequate.

The intravenous administration of oxygen to patients is not new. It has been reported several times. In the past, however, technical difficulties with the apparatus available prevented the use of this method of oxygen administration.

In this preliminary report is described an apparatus which is now being used for the intravenous administration of oxygen. The results are highly satisfactory and further work, including case reports, will be made the subject of later papers.

In 1902 Mariani[1] injected 120 c.c. of oxygen in forty-five minutes into a dying tuberculous patient (rate of 160 c.c. per hour). He reported that the pulse rate and respiration were improved. The treatment was not repeated. The patient died the next day.

Tunnicliffe and Stebbing[2] in 1916 treated three very ill patients with oxygen intravenously. Their apparatus consisted of a cylinder of "Brinn's oxygen," which contained, they thought, about 1.5 per cent of nitrogen; an "endurance regulator"; pressure tubing; a piece of glass tubing drawn out at the end; a wash bottle and a screw clip on the tubing. The wash bottle contained a solution of 2 per cent sodium carbonate, part of which was blown out by the stream of oxygen and part remained to wash the remaining stream. The amount of oxygen given was estimated by counting the number of bubbles per minute coming through the wash bottle. These authors state that oxygen can be given at the rate of 600 to 1,200 c.c. per hour, that cyanosis and dyspnea are rapidly relieved, and further: "The beneficial results of the intravenous injection of oxygen were certainly more permanent than could apparently be explained by the mere relieving of the cyanosis." The work done with patients fully confirms this observation. Tunnicliffe and Stebbing conclude their paper as follows: "The object of this preliminary note is not to indicate the specific pathological conditions in which the intravenous injection of oxygen should be used, but simply to point out that in those cases in which

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the inhalation of oxygen either cannot be practiced or gives inadequate relief, the intravenous method of oxygen administration, if carefully carried out in accordance with the directions, is available to the clinician and will give therapeutic results."

Singh and Shah,[3] of the Haffkine Institute in Bombay, in 1940 gave oxygen to six patients with severe pulmonary disease. These workers used two different methods. The first consisted of two syringes, moved by screws, working alternately, to pump the oxygen into the vein. There was a pause of five to ten seconds between the actions of the plungers in the syringes. (This allows blood to flow into the end of the needle in the interval when oxygen is not coming through, increasing the danger of clot formation in the needle. Such a clot, if formed, would be dislodged into the venous blood stream when the oxygen flow is resumed.) The second method was similar to that of Tunnicliffe and Stebbing,[2] which they found was hard to control and was apt to produce pulmonary embolism. With the first method they incorporated a manometer to register the venous pressure, which also indicates any stoppage in the flow of oxygen.

Singh and Shah[3] found that the signs and symptoms of pulmonary gas embolism in man are the same as those in animals. They state: "With slight embolism there is a rise in blood pressure and pulse rate; this does not indicate any danger, and the oxygen should not be withheld. With a greater degree of embolism the blood pressure falls, the pulse becomes feeble, and cyanosis increases. The patient complains of a sense of impending dissolution, uneasiness over the chest, shortness of breath and pain over the precordial region. Oxygen embolism, however, unless carried to extreme is not dangerous." The above authors further state, "all these (six) cases showed clinical improvement for 24 to 48 hours after injection, as indicated by raised blood pressure and improved pulse."

Summarizing their report, Singh and Shah[3] state: "In man about 10 to 20 c.c. of oxygen a minute (600 to 1,200 c.c. per hour) can be administered intravenously; this is about 10 per cent of the basal requirement. This is the only method by which any considerable quantities of oxygen can be administered by an extrapulmonary route. This amount of oxygen does not appear to be considerable, but still a distinct clinical improvement follows its administration."

The work with animals has been more extensive, but it has, on the whole, not been performed carefully enough and has been very misleading for technical reasons, which will be pointed out.

The first workers to inject oxygen intravenously were Nysten[4] in 1811 and Demarquay[5] in 1866. They used dogs.

Gaertner[6] in 1902 also gave oxygen intravenously to dogs. He stated that it did not obstruct the circulation through the lungs. He found bubbles of gas in the right ventricle but never in the left; he contended dangerous symptoms can immediately be relieved by withholding the oxygen. In 1904 Stuertz[7] injected oxygen intravenously in dogs. He recommended intermittent injections and minimized the possibility of embolism. These injections constitute one of the technical faults which have, in the past, led to the abandonment of this form

of therapy. Intermittent injections cause (1) clotting in the needle, or (2) frequent withdrawal of the needle, and as frequent subsequent venipunctures.

More recent experimental work with animals has apparently been more carefully performed, especially with reference to the measurement of the amount of oxygen administered in relation to body weight. The recent work, however, incorporates practically the same technical errors present in the older methods.

Bourne and Smith[8] in 1927 performed experiments with dogs. They state: "It is seen, however, that in amounts (of oxygen) that would provide some hope of the supply of an adequate amount, pulmonary embolism and right heart insufficiency produce an even greater degree of anoxemia than that already in existence." Here lies the cardinal reason for the failure of former investigators to make the intravenous administration of oxygen successful. It has always been *assumed* that it would be necessary to give an amount of oxygen which would approximate the total or basal requirement of the body. This concept we have found to be erroneous. The fact has been overlooked that the body is (even in severe anoxemia) getting *some* oxygen through the lungs.

Bourne and Smith[8] gave dogs from 0.75 c.c. to 3 c.c. of oxygen per kilogram per minute. This corresponds to a rate of 3,150 c.c. to 12,600 c.c. of oxygen per hour for an average 70 kg. man. In contrast, I have obtained favorable results in man by the intravenous administration of from 200 to 1,000 c.c. per hour, and have given oxygen at these rates for periods up to nine hours continuously.

Macdonald Dick[9] in 1939, following the lead of the afore-mentioned workers, gave oxygen to dogs in the femoral vein. He administered 2 to 3 c.c. of oxygen per kilogram of body weight per minute "with safety" and stated that "much larger amounts can be given for short periods." Converting these amounts to values for an average 70 kg. man, we find that they amount to 8,400 c.c. to 12,600 c.c. per hour. Again, too much oxygen was given. In fact, the amount given was ten times that which has been found to be therapeutic.

In the past the failure to obtain beneficial results in a practical manner has been due to several factors. Former workers were under the influence of a great curiosity as to the effects of embolism. They made it a point to give so much oxygen that they could observe the effects of severe oxygen embolism.

Animal experimentation as performed up to this time has exhibited several other defects. Most workers with animals have neglected or considered it unnecessary to produce oxygen want. They have not used apparatus which could supply a constant flow of oxygen at the low rate of flow required.

When too much oxygen is administered by the intravenous route, the difficulty is not due to embolism in the ordinary sense. It is due to what is called *tampon*, caused by blood froth in the lungs and right heart. This phenomenon is also called *vapor lock*. These difficulties are no longer of any importance when oxygen is administered intravenously at a constant volume and pressure, each of which is carefully controlled. This is possible with the apparatus described below.

THE APPARATUS

Any apparatus used for the intravenous administration of oxygen must meet certain definite requirements. It must be easily regulated. The amount

of oxygen which is delivered must be directly indicated by a manometer scale calibrated in cubic centimeters of oxygen per hour. The volume of oxygen delivered by the apparatus should range between 100 and at least 1,200 c.c. per hour. The flow of oxygen must be continuous and not intermittent. The apparatus must also be so constructed that the flow of oxygen will not be stopped by an increase in the venous pressure. The apparatus consists of the following:

A tank of medicinal grade oxygen, which should be at least 99.5 per cent pure. Such cylinders of oxygen are commercially available. The size of the oxygen tank may be whatever is convenient; a small tank, such as a 20 or 40 gallon tank, would be relatively light, take little space, and could be readily transported.

Attached to the oxygen cylinder by a yoke is a double pressure reducing valve. Attached to the yoke is an indicator showing the pressure of the oxygen in the tank. The first of the pressure reducing valves is a fixed valve requiring no adjustment. This valve reduces the high pressure in the tank to a low level approximating some convenient low pressure, such as 5 pounds per square inch.

The second of the pressure reducing valves through which the oxygen must flow is adjustable. This valve must be so constructed that it will deliver a pressure ranging from zero up to about 200 mm. of mercury, as set. The spring and screw which regulate this valve must be so designed that the pressure can be easily adjusted with an accuracy of 1 to 2 mm. of mercury or better. The outlet from the adjustable pressure reducing valve must have a manometer attached to it to indicate the reduced oxygen pressure. This manometer can be of the mercury type or the so-called aneroid or spring type. Whichever type of manometer is used, it should be calibrated in millimeters of mercury from zero to at least 200 mm., it must also be calibrated to show the flow of oxygen in cubic centimeters per hour.

From the outlet of the second pressure reducing valve the oxygen is conducted by tubing to a filter which contains a convenient number of layers of cotton and calcium chloride or anhydrous calcium sulfate, or some other efficient dehydrating substance. The purpose of this filter is to make sure that the oxygen will be dry. From the filter the oxygen is conducted through more tubing to a small chamber containing a resistor.

This resistor may consist of a porcelain block or a block of some other suitable porous substance, such as porous bronze. The pressure of the oxygen will force it through this porous block. The rate of flow of the oxygen will be directly proportional to the difference in pressure between the oxygen on the proximal side of the porous resistance block and the pressure of the oxygen on the distal side of the porous resistance block, multiplied by a factor which will be constant for any given resistance. This latter factor would have to be determined by the calibration of each apparatus constructed.

Incidentally, the dehydrating filter and the porous fixed resistor would each remove any foreign particulate matter from the oxygen flowing through the apparatus if any was present.

From the housing containing the porous resistor, the oxygen is conducted by tubing to two places. First, the oxygen is conducted back to the mercury or aneroid manometer, but to the opposite side of the manometer, so that the manometer shows directly the difference between the pressures on each side of the fixed resistance. Second, it is conducted through tubing to a wash bottle where the oxygen bubbles through a liquid such as distilled water. This makes the flow of oxygen visible and humidifies it.

From the wash bottle the oxygen is conducted through a suitable outlet to which is attached in each case, before the apparatus is used, a sterile outfit consisting of a sufficient length of rubber tubing, a small two-way valve so that a syringe may be attached to the tubing, a glass adapter, and a needle for injecting the vein. I have adopted a 22 gauge needle.

To use the apparatus the oxygen is turned on and allowed to flow for a reasonable time so as to wash out any residual air. While the oxygen is flowing, the needle is inserted into the patient's vein.

When a tourniquet is used to distend the veins, it causes an appreciable increase in venous pressure. Therefore, when the needle is first injected, and before the tourniquet is released, a small amount of blood may flow into the needle. However, when the tourniquet is released, the oxygen pressure immediately pushes the blood back into the blood stream. There is a small amount of blood remaining which adheres to the surface of the lumen of the needle. This blood may be washed out of the needle by attaching a syringe containing a solution of sterile sodium citrate or other suitable anticoagulant solution of suitable strength to the small two-way valve. The valve is so adjusted that the citrate solution can be injected from the syringe into the rubber tubing just proximal to the glass adapter. The valve is then adjusted to cut off the flow from the syringe and to allow the oxygen to flow through the sterile rubber tubing. The oxygen coming through pushes the sterile citrate solution through the glass adapter and the needle, washing out any blood which may be present. This process of adding citrate solution to the tubing can be used previous to the initial injection of the needle into the vein.

It is also recommended that the apparatus have a manometer, preferably of the aneroid type, with a range from zero to about 60 mm. of mercury. This manometer is attached by tubing to the apparatus at some point between the fixed resistance block and the sterile rubber tubing. It is suggested that it be attached so as to show the pressure of the oxygen in the wash bottle. This pressure will correspond very closely to venous pressure.

Normal venous pressure ranges between 4.4 and 8.8 mm. of mercury. In heart failure venous pressure will rise from about 9 mm. of mercury to as high as 30 mm. of mercury in severe grades. If there is any stoppage in the apparatus, such as would occur if a clot were to form in the intravenous needle, this would be indicated by an unusual rise in pressure, as shown by the gauge. This gauge also provides valuable clinical information as to the degree of heart failure.

The outlet in the wash bottle from which the oxygen bubbles must have a small aperture. If this aperture is large, the bubbles coming through will be large and will cause a "bump effect," which is really a slight variation in the

pressure of oxygen. This effect may cause an uneven flow of oxygen into the vein. The apparatus must be so constructed that the oxygen flow is always uniform.

One should use a new stainless steel needle, entirely free of corrosion, or a gold or platinum needle. See Figs. 1 and 2 for further details.

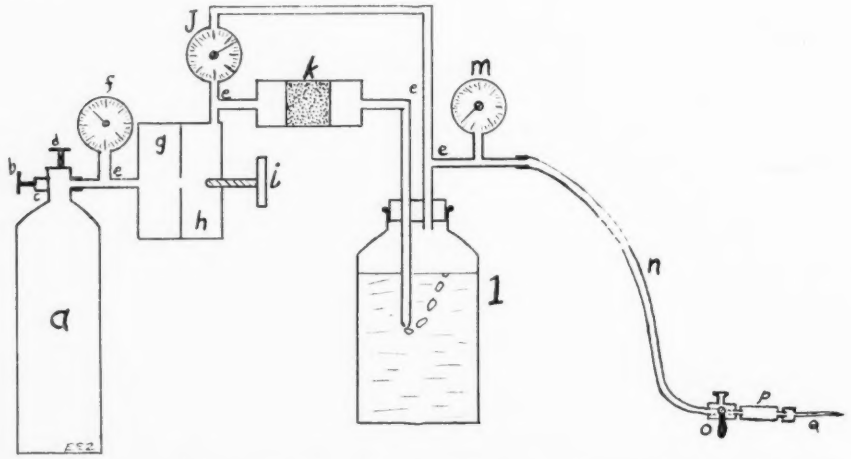

Fig. 1.—Apparatus for intravenous oxygen therapy. Schematic drawing: *a*, tank of compressed oxygen; *b*, screw for fastening yoke to tank; *c*, yoke; *d*, valve for opening tank; *e*, tubing; *f*, gauge showing tank pressure; *g*, fixed pressure reducing valve; *h*, adjustable low pressure reducing valve; *i*, screw for adjusting oxygen pressure and volume of flow; *j*, manometer showing volume of oxygen flow; *k*, fixed porous resistance; *l*, wash bottle; *m*, venous pressure gauge; *n*, *o*, *p*, *q*, sterile tubing, two-way valve, and adapter for receiving glass syringe, glass adapter, and intravenous needle.

The apparatus I have devised always operates under positive pressures of oxygen without interruption or variation, except as adjusted. Any leak developing in this apparatus could not possibly admit air, as oxygen would escape from the apparatus to the outside, preventing air from gaining access to the apparatus.

An additional advantage of this apparatus is that it always supplies oxygen by pressure which is slightly greater than that of the venous pressure. If the venous pressure varies, the pressure in the apparatus will vary accordingly, always remaining slightly higher than the venous pressure. Other types of apparatus that have been used experimentally and that have not been practical for therapeutic work have supplied oxygen at pressures determined by the characteristics of the apparatus without respect to the venous pressure.

Another advantage of the apparatus is that it requires no pump device or electric motor. The oxygen proceeds through the apparatus by virtue of the pressure of the oxygen in the compressed oxygen cylinder. Thus the apparatus can be used at any place, whether or not there is a source of energy, such as electric power.

CLINICAL CONSIDERATIONS

Cases will be reported in detail later. A large variety of disease conditions in which oxygen therapy is indicated has not been available up to this time. However, the patients who have been treated have shown in general the following results: Cyanosis is abolished. The pulse rate becomes lower.

The blood pressure, if low, tends to rise. The mental state becomes clearer. One patient who was comatose became conscious within a few minutes. One who had severe pulmonary edema was entirely relieved of the edema after twelve hours, following a five-hour intravenous administration of oxygen.

Various comments have been made voluntarily by patients to the effect that they preferred this form of oxygen therapy to others which they have experienced.

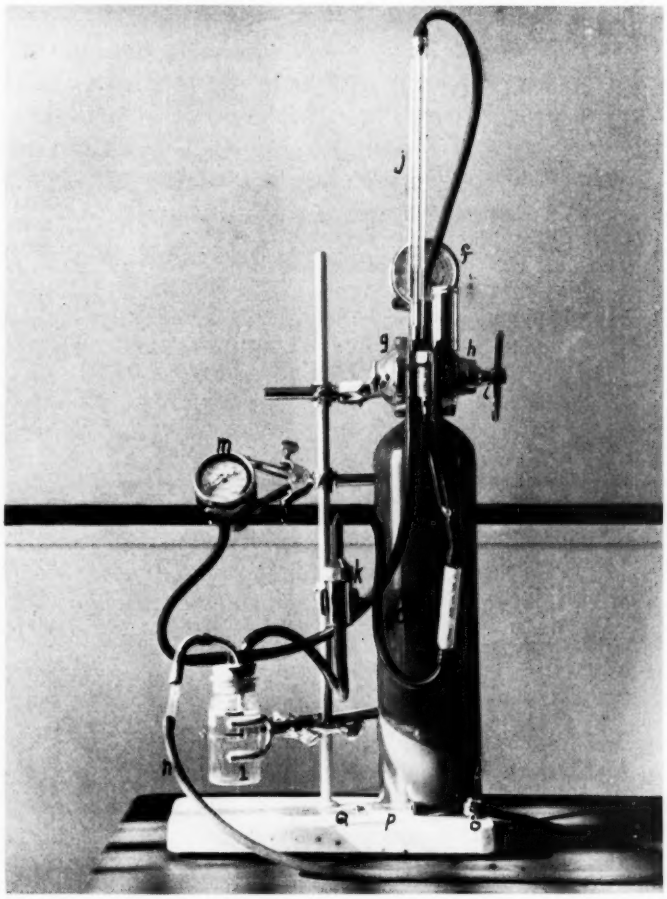

Fig. 2.

The treatment is no more complicated than the administration of solutions intravenously, and the apparatus requires but little more attention. The nursing care of the patient is much easier than when he is in an oxygen tent. No unfavorable reactions, subjective or objective, have been observed thus far.

I have not had the opportunity to study the signs and symptoms of pulmonary oxygen embolism, which others have observed, because I do not give enough oxygen to cause pulmonary embolism. According to Singh and Shah,[3] the signs and symptoms of pulmonary gas embolism (tamponade) are the same as those observed in animals. These authors state, "With slight embolism there is a rise in blood pressure and pulse rate; this does not indicate any

danger; and the oxygen should not be withheld." With this statement I cannot entirely agree. I believe that if an amount of oxygen is being given, which causes a rise in blood pressure with a corresponding rise in pulse rate, the flow of oxygen should be reduced in amount. The signs and symptoms of pulmonary oxygen embolism apparently furnish adequate warning of an overdose.

In my experience, using therapeutic amounts of oxygen, averaging 200 to 600 c.c. per hour, I have observed that when the blood pressure is low, as in shock or heart failure, it will rise. There is also an accompanying slowing of the pulse rate and a less labored and a slower respiration. *Thus the pulse rate and the respiratory rate are the important things to observe during the intravenous administration of oxygen.* An appreciable increase in pulse rate and respiratory rate occurring during the administration of oxygen would indicate that the rate of oxygen administration was too great.

ECONOMICS

On a twenty-four-hour basis the cost of the oxygen administered intravenously would not be over ten cents, and would probably be somewhat less. Contrasted to this, the cost of oxygen, on a twenty-four-hour basis, as administered by tent might range anywhere from $6.00 to $20.00. The original cost of the apparatus would be much less than the average type of apparatus used at the present time for administering oxygen by other routes.

DISCUSSION

It is my belief that the intravenous administration of oxygen is more effective and very much less expensive than other methods of oxygen therapy.

The improvement observed in patients seems to be all out of proportion to the small amount of oxygen administered. This is rather difficult to explain in exact scientific terms. But an explanation is offered as follows:

When oxygen is administered intravenously, the body is getting something that it urgently needs without expending any energy. It requires a certain amount of energy for the pumping action of the heart and the respiratory movements of the chest to obtain oxygen for the use of the tissues. Any oxygen given intravenously reduces the amount of work thus required of the heart and chest. In addition to this, the physiologic processes of the body, in general, seem to be improved, no doubt as a result of improved tissue metabolism.

When oxygen is given intravenously, it provides opportunity for reduced hemoglobin to absorb oxygen directly. This direct absorption of oxygen by hemoglobin can occur in the vein, in the right side of the heart, and finally in the vessels of the lungs. That this absorption does occur is shown by the clinical improvement which results.

If one listens with a stethoscope over the tricuspid area of the chest during the intravenous administration of oxygen, one hears bubbles of oxygen rushing about in the right side of the heart. If one then listens over the mitral area of the chest, no bubbles can be heard. This is an indication that the oxygen is absorbed before it reaches the left side of the heart.

Another possibility remains; this is, that the oxygen, or a portion of it, may not all be absorbed by the venous blood and may pass from the lung capillaries into the alveoli of the lung. On the grounds of clinical evidence I do not believe that this happens. Dick[9] studied this point and states, "it should be noted in this connection that the oxygen consumption of the animal remained unaltered, the oxygen introduced by vein being compensated for by a decreased absorption from the lung." This finding, based on careful work done by Dick, was in spite of the fact that he gave approximately ten times as much oxygen as I have found necessary for therapeutic results.

The clinical evidence of improvement is perhaps not sufficient to convince all of the value of this method of treatment. It is proposed to measure the oxygen consumption with a metabolism apparatus before and during the intravenous administration of oxygen in a manner similar to that used in animal work by Dick.[9] Another laboratory method which I expect to use is the analysis of arterial blood for its oxygen content before and during the intravenous administration of oxygen.

It is believed that the effect of the intravenous administration of oxygen should be determined in certain types of mental disease, such as alcoholism and dementia praecox.

Another brief discussion of oxygen embolism is in order. The phenomena of air embolism are produced by nitrogen. Bubbles of nitrogen present in the capillaries of a tissue will shut off this tissue from its supply of oxygen. In contrast to this, bubbles of oxygen in the capillaries of a tissue cannot shut off this tissue from its supply of oxygen. Animal and human experiments indicate that tissues will gradually absorb these bubbles of oxygen. When this happens, the blood flow through the capillaries is again re-established, and the tissues in the meantime have not been deprived of oxygen.

I have been informed that medicinal grade oxygen is 99.5 to 99.8 per cent pure. The impurities present are said to consist mostly of nitrogen with slight traces of other inert gases. From what I know of the elimination of nitrogen in caisson disease, it can be assumed that this amount of nitrogen is of no significance. In general, those who have employed oxygen intravenously in the past have not had available a supply which was 99.5 per cent pure.

Two contraindications to the intravenous administration of oxygen have been discovered. When there is a marked diminution in the capacity of the vascular bed of the lungs, such as occurs in advanced tuberculosis and emphysema, the amount of oxygen given intravenously should be greatly reduced in amount, or not given at all.

When there is a marked dilatation of the right ventricle, as shown by an unusually high venous pressure, intravenous oxygen may be of no avail. The above conditions are obviously related, since increased resistance to the flow of blood through the lungs is the usual cause of dilatation of the right ventricle.

The apparatus presented may be used experimentally for the intravenous administration of gases other than oxygen, such as gaseous anesthetics, gas-oxygen mixtures, and other gases.

SUMMARY

An apparatus has been devised and described which makes the intravenous administration of oxygen safe.

The intravenous administration of oxygen to patients has, in my experience thus far, been more effective than oxygen administered by any other routes.

The cost of the administration of oxygen intravenously is almost insignificant in comparison with administration by other methods.

It is believed that the intravenous administration of oxygen can be used in most of the conditions in which oxygen therapy is indicated.

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CULTIVATION OF *SPIROCHAETA PALLIDA* ON THE CHORIO-ALLANTOIC MEMBRANE OF THE DEVELOPING HEN EGG*

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MANY attempts have been made to cultivate *Spirochaeta pallida* on artificial media.[1-7] At present, however, infected rabbits are the only reliable source of the organisms for experimental purposes. To obtain fairly large quantities of spirochetes more conveniently, and possibly more quickly, we have attempted their cultivation on the chorio-allantoic membrane of the developing egg. Sterzi and Staudacher[8] have reported survival of the organisms on the chorio-allantois of the egg for six hours, and retention of virulence for two hours, using the Truffi strain of *S. pallida*.

In our work the Nichols strain† was used directly after one rabbit passage. The material used in the beginning was a frozen 50 per cent testicular mince suspended in 1 per cent neopeptone veal infusion broth, pH 6.9.[9] The mince was thawed rapidly in a 37° C. water bath, and 0.25 c.c. was injected into the right testicle of a healthy male rabbit. The animal showed characteristic lesions of rabbit syphilis thirty-nine days after inoculation. A dark field of the testicular fluid revealed many motile spirochetes. The rabbit was sacrificed by injecting 20 c.c. of air into the external vein of the left ear. The testicle

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†This material was kindly furnished by Dr. W. L. Fleming, of the Division of Public Health, University of North Carolina.

was excised, cut into pieces of about 5 c.mm., and ground for ten minutes with sterile sand. One per cent Difco peptone beef infusion broth, pH 7.4, was used to make a 50 per cent suspension by weight of the testicular material. The suspension was well mixed, and the sand and heavy tissue were thrown down by centrifuging at low speed (350 r.p.m.) for five minutes.

Fig. 1.—Rabbit No. 15, fifty-nine days after inoculation.

Using the Goodpasture technique,[10] 0.1 c.c. of the supernatant fluid was dropped onto the chorio-allantoic membrane of each of ten fertile hen eggs which had been incubated ten days at 38° C. After inoculation the eggs were returned to the 38° C. incubator. The time elapsed from the rabbit's death to inoculation of the eggs was one and one-half hours.

One of the embryos ceased normal movement seven hours after inoculation. At this time, 0.25 c.c. of its chorio-allantoic fluid was injected into each testicle of a healthy male rabbit (No. 14). The material injected showed a few feebly motile spirochetes by dark-field examination. However, no syphilitic lesions have been observed in the rabbit in ninety days.

A second embryo was dead twenty-seven hours after inoculation. This egg was opened, and 0.25 c.c. of its chorio-allantoic fluid was injected into each testicle of another healthy male rabbit (No. 15). Dark-field examination showed one or two sluggishly motile spirochetes per field. Forty-seven days after inoculation the right testicle of this animal was swollen and indurated, the left testicle remaining normal in appearance. Fifty-nine days after inoculation both testicles were greatly swollen and hard masses could be felt. There was a chancre-like lesion 2 cm. in diameter on the left testicle (Fig. 1), and numerous motile spirochetes could be seen in dark-field preparations.

On the sixty-fourth day following inoculation, rabbit No. 15 was sacrificed; both testicles were cut and ground with sterile sand, then mixed with Difco peptone beef infusion broth, pH 7.4, to make a 50 per cent emulsion by weight. The supernatant liquid was prepared by centrifuging as before, and 0.5 c.c. of it was used to inoculate another rabbit (No. 19). At this time, the preparation showed many motile spirochetes by dark-field examination. The remainder was frozen and preserved at –78° C.

Tissue sections of the liver and heart of the embryo from the egg used to inoculate rabbit No. 15, stained by Levaditi's method, showed no spirochetes. The remaining eight of the ten eggs inoculated were allowed to hatch, and the chicks appeared normal for ten days, the limit of observation. Tissue sections of their hearts and livers taken at this time showed no recognizable spirochetes.

Twenty-four days after inoculation rabbit No. 19 showed swelling and induration of both testicles. Thirty days after inoculation dark-field preparations of material aspirated from each testicle showed numerous motile spirochetes.

The infection of rabbit No. 15 indicated that virulent spirochetes of the Nichols strain will live twenty-seven hours in the developing hen egg and, in some cases, will retain their virulence for that length of time. The infection is transferable from one rabbit to another, the first rabbit-to-rabbit transfer producing rabbit syphilis within the normal time limit. More work is necessary to determine how great a percentage of the egg inocula will live, whether spirochetes will multiply in the developing egg, and in which part of the egg surviving or growing spirochetes are most abundant.

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TENSILE STRENGTH OF HAIR AND HAIR ROOTS

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IN THE preparation of animals for intradermal or subcutaneous injections, the usual practice is to shave the dry or lathered hair with a razor, sometimes after preliminary clipping with a scissors or hand or electric clipper.[1] Occasionally a chemical depilatory, such as barium sulfide, is used. At the Olive View Sanatorium it has been found simpler to pull out the hair from the guinea pigs in about half a dozen tufts, thus clearing a space large enough for the injection. This plucking is faster, lasts longer, and produces less local irritation. Only a few seconds are required, as compared with the longer time needed for clipping, lathering, shaving, and getting, cleaning, and replacing the clippers and razors, or applying and removing the depilatory. The part which is plucked remains bare for many days longer than the part which is shaved, thus allowing better observation of local reactions that may occur. With the plucking of hair, the guinea pig skin is left smooth and pale, and no inflammatory reaction follows, so that the reaction to injections may be readily noted. On the other hand, shaving is not only followed by gross cuts and injuries, but microscopic examination of the freshly shaven skin indicates that many subvisible lesions may also be inflicted, any of which increases the chances for local irritation, inflammation, and infection.

Insofar as sensation or distress can be gauged by the behavior of the animals, it appears that plucking is less painful to the guinea pigs than the more prolonged procedures of shaving or applications of epilating pastes. The guinea pig hair usually comes out so readily that there is not the tension on the skin, which is the usual cause for pain in hair-pulling. Guinea pigs seem much more frightened than hurt by the procedures in general, and soon become used to handling. They usually utter the greatest amount of audible complaints, squeals, etc., while they are being sought and picked up, subsiding soon after they are firmly grasped. If not too great a tuft of hair is taken at each time, and the plucking is done rather brusquely, they may make no further protest during the entire procedure, or at most only a single yelp at the first touch. With a little practice technicians become so adept at this mode of epilation that they can prepare a large number of animals for testing within a few minutes, with practically no complaint on the part of the subjects.

The ease with which hair may be plucked from guinea pigs is markedly in contrast with the resistance to such hair-pulling shown by rabbits and other animals, especially the fur-bearing species. For such subjects, shaving offers a much better means of baring the skin. Very simple observations were suf-

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ficient to demonstrate that this was the case. Even the removal of a few hairs from a rabbit is difficult; to pluck a large clump of hair may result, if sufficient force is used, in actual disruption of the skin, if the hair does not, as is more often the case, break in the grasp.

In order to obtain quantitative data regarding the ease of removal of hair from the guinea pig, a series of simple measurements was undertaken. A light wire spring scale was calibrated by attaching various small weights, from 1 to 100 Gm., to a mosquito forceps at its lower end. When the forceps grasped a hair instead, and the spring scale was withdrawn, the elongation of the spring and the corresponding tension exerted were readily observed.

Ordinary human hair, pulled from the forearm of an adult male, exerted a tension of from 5 to 25 Gm. before extraction, with an average of 12 Gm. per hair. Thereafter, pulling at the extracted hair until it broke off somewhere in its length required another force of from 8 to 32 Gm., with an average of 18 Gm. per hair.

Accordingly, a human hair will usually, when pulled sufficiently, come out from the skin, but if jerked rapidly, it is apt to break off, since the breaking stress is about 50 per cent greater than that of epilation.

On the other hand, hair from the back of a guinea pig came out with the application of from 1 to 5 Gm. force, usually about 2 Gm. However, the same hair could not then be broken without the application of from 10 to 52 Gm. of tension, averaging about 25 Gm. It appears, then, that the hair of the guinea pig is really somewhat stronger than that of man, but that the hair root in the guinea pig is quite fragile, releasing the hair at less than one-sixth the tension required to pull out a human hair, and less than one-twelfth the force required to break off the hair. Accordingly, the chances of breaking off the hair on plucking a guinea pig, instead of extracting it, are very slight.

Further studies in the same manner may be desirable to elucidate the effect of season, sex, age, pregnancy, hair, and skin color, hair length and thickness, curliness of hair, etc., as well as the effects of various diets, diseases, and environmental factors on the hair of different species.[2]

The present study merely documents the previously observed fact that the hair of the common guinea pig is readily removed by simply plucking it out in clumps, and that this makes a much simpler and more effective way of epilating a guinea pig than does shaving, clipping, or the application of depilatory paste or lotions.

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AN INEXPENSIVE ANIMAL COMPRESSION CHAMBER*

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IT IS possible to construct a simple and efficient compression chamber for small animals at very low cost. The chamber described here was built in the school shop at a cost of less than $15.00 and has given almost uninterrupted service for four years. It was originally used in animal experiments incidental to the development of helium-oxygen mixtures for use by divers and others working under increased atmospheric pressure.[1] It can be used to subject rats, mice, guinea pigs, and small rabbits to increased pressure in any gas or mixture of gases. By substituting an appropriate gauge and using suction instead of high pressure, it may be employed in experiments in reduced pressures as well.

The body of the chamber consists of a 1-foot section of 6 inch standard steel pipe, carefully squared on the ends. Next, a circular steel plate 10 inches in diameter and ⅝ inch in thickness is converted into the door or back cover of the chamber. This plate is centered in a lathe, and a shallow, circular trough is cut into one face. The trough is just wide enough to accommodate the end of the 6 inch pipe comfortably. Later this trough is filled with melted lead to form a gasket. The center is then cut out of a second plate of the same size. The ring thus produced is just large enough to slide over the outside of the chamber and form a collar for it. This collar is later welded in place 1 inch from the back end of the chamber. A third plate of the same size has a 4 inch circle cut from its center, with the cut beveled ¾ inch toward the outside. This last plate covers the window of the chamber and holds it tightly in place. The window itself consists of a 6¾ inch circle of plate glass 1 inch in thickness.

The various parts of the compression chamber are held together by eight steel rods made from eight 16 inch lengths of ¾ inch mild steel rod, threaded for a distance of 1½ inches on one end and for 3½ inches on the other. These rods pass through holes drilled in the collar and end plates of the chamber. First the two end plates are drilled with eight 13/16 inch holes spaced evenly around the margin, with their centers ⅞ inch from the edge. Using these plates as a template, the collar is then drilled with eight holes which are tapped to take the threaded rods. The rods are all threaded through the collar from one side, using the ends which had been threaded for a distance of 3½ inches. The collar is then easily held in position while being welded to the chamber by placing the two end plates in position over the ends of the rods and drawing them up snugly by means of nuts.

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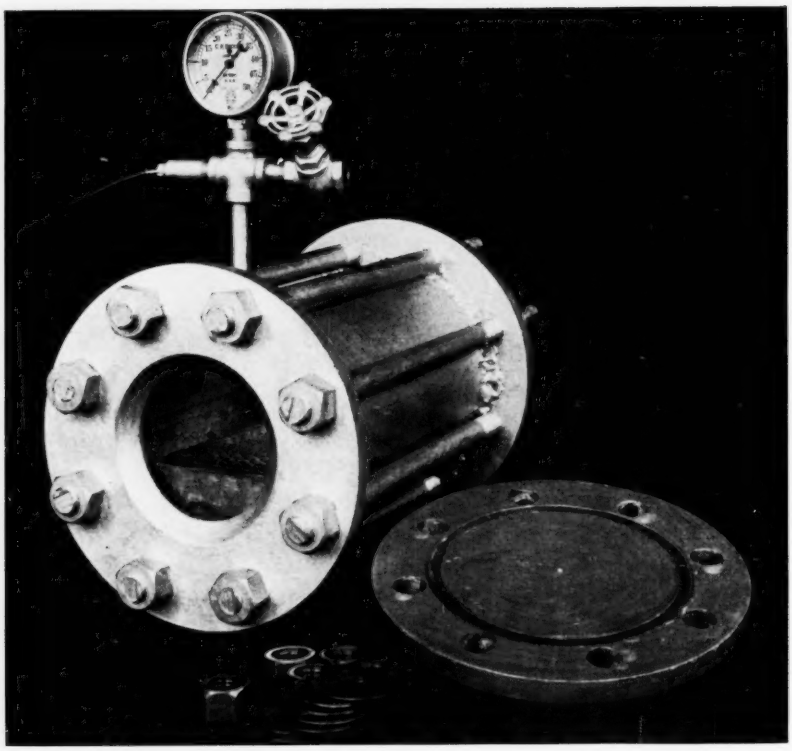

Fig. 1.—Compression chamber showing view through window; method of attaching gauge and valve; and back cover removed to show lead gasket.

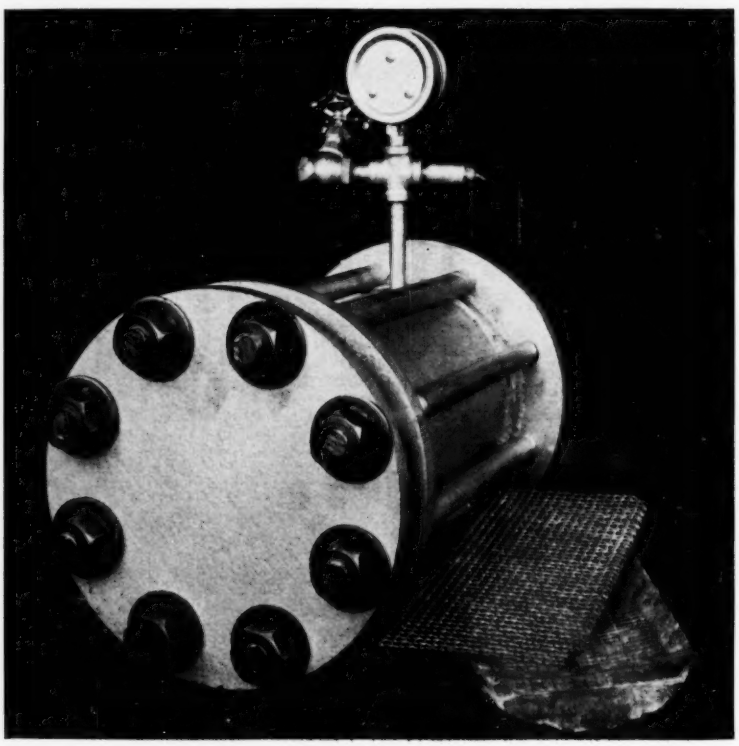

Fig. 2.—Back view of chamber with cover in place. The tray for chemicals and the screen floor of the chamber are shown.

The chamber is assembled by placing a thin, paint-soaked leather gasket between the end of the pipe and the window, and a plain gasket between the window and the plate which covers it. This plate is then drawn up very tightly and evenly by means of nuts on the ends of the ¾ inch rods. The cover of the chamber is tightened in place in the same way. The cover and one of the rods are punch marked to insure replacing the cover in the same position every time. After the nuts have been tightened and released several times, the lead gasket becomes shaped to the end of the pipe and forms a tight joint when the cover is drawn up tightly and evenly.

The chamber is completed by drilling a hole through the top and threading into it a short piece of heavy ½ inch brass pipe, to which is attached a brass cross. A pressure gauge is then attached to the vertical arm of the cross, and a needle valve to each side arm. Such an arrangement permits the introduction of gas through one side arm and its subsequent removal through the other one. Also, two gases or mixtures of gases can be used by introducing one from each side. As can be seen from Figs. 1 and 2, a packing box has recently been substituted for one of the needle valves to permit an insulated wire to be run into the chamber to provide current for illumination whenever it is desired to photograph the animals.

Carbon dioxide and moisture may be eliminated by ventilating the chamber; a galvanized iron tray of chemicals beneath the screen floor assists in this elimination. The bottom of this tray is filled with calcium chloride to absorb moisture. A blotter saturated with concentrated sodium hydroxide solution, to absorb excess carbon dioxide, is supported on a screen above the tray. The floor of the chamber is made of heavy screen placed high enough above the blotter to prevent any caustic from coming in contact with the animals.

The chamber before being used for experiments should be tested by filling it with water and then subjecting it to a pressure considerably higher than any that will be used experimentally. A cylinder of compressed air provides a very convenient source of high pressure for this test. Leaks will be betrayed by the escape of water, and no violent explosion will occur if any part breaks. It is theoretically safe to employ pressures of several hundred pounds per square inch in this chamber, but we have never had occasion to use pressures in excess of 100 pounds. If high pressures are used, a mirror should be arranged to permit the observer to see the inside of the chamber without standing in front of the glass window itself.

SUMMARY

The construction of an experimental compression chamber at a cost of less than $15.00 is described. In this chamber rats, mice, guinea pigs, and small rabbits can be subjected to pressures in excess of 100 pounds per square inch in any gas or mixture of gases, and their reactions can be observed. The chamber may be employed in studying the effects of reduced pressure.

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A DIRECT PLETHYSMOGRAPHIC METHOD FOR DETERMINING THE BLOOD PRESSURE IN THE UNANESTHETIZED RAT*

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WITH the development of techniques for producing hypertension in the rat, many investigators have attempted to devise methods for the indirect measurement of blood pressure in small rodents. Early methods made use of anesthetized animals,[1] but since the anesthetic itself had some effect on the blood pressure, methods were sought for use on the nonanesthetized animal. The first of these was that of Diaz and Levy,[2] which gave reliable results but necessitated the cutting of the tail and some loss of blood. Williams, Harrison, and Grollman designed an ingenious apparatus which, in their hands, gave very consistent results.[3] In the course of our investigations this method was used with some success, but we encountered several difficulties. In the first place, the method requires that the animals be warmed and trained, and is not always feasible. Furthermore, there is no objective criterion by which to determine whether the animals have been equally warmed. In addition, we found that since the tail is separated from the water in the water chamber by a surrounding thin rubber tube, the apparatus is not very sensitive to small variations in pressure, and works best with a rat whose tail fits close to the rubber tube. In the case of small rats it is not always possible to collapse the tube about the tail without leaving air spaces, and as a result the expansion of the tail at systolic pressure may be taken up by the slack of the rubber tube.

To obviate these difficulties we designed the following apparatus which employs direct plethysmography, and requires neither training nor preliminary heating. In our hands this apparatus has given very consistent results.

DESCRIPTION OF THE APPARATUS (Fig. 1.)

Part 1 consists of a cylinder 2 inches in diameter and 6 inches long, made of continuously perforated tin or other metal. This is closed off at *a* by a cork which can be used to adjust the tube length. The other end is closed by a sleeve cylinder of metal made to fit over the tube (about 2⅛ inches in diameter). This sleeve is completely closed at *b* except for a ⅜ inch hole drilled in the center to permit passage of the tail. This part holds the animal.

Part 2 consists of a glass cylinder of 1 inch diameter and ½ inch long, closed at both ends except for the flanged apertures at *c* and *d* as shown. These are ⅜ inch and 5/16 inch, respectively. At e there is a short glass tube of 1/16 inch bore to admit air. Cigarette drainage tubing of ⅜ inch diameter is passed through the two apertures, stretched and cemented over the flanges, and tied down. This completes the pressure cuff. For small rats of less than 100 Gm. a similar cuff is used, but with apertures of ¼ inch and 3/16 inch, respectively.

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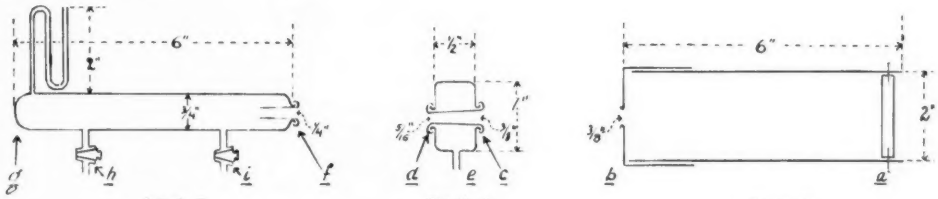
Part 3 is a cylinder of glass, ¾ inch in diameter, with a flanged opening of ¼ inch at f, and closed at g. The tube length is 6 inches. At the superior surface of the distal end an S-shaped capillary manometer (0.5 mm.) is placed, which rises 2 inches above the tube. At *h* and *i* stopcocks are placed for water inlet and outlet. A one inch length of ¼ inch cigarette drainage tubing is cemented in place over the flange, which when snugly applied to the tail acts as a stopper for that end of the tube. This diameter of tubing is most satisfactory for overall use, while 5/16 inch will be found better for larger animals (above 150 Gm.) and 3/16 inch for small animals (less than 100 Gm.). The design of the apparatus permits replacing or changing of the rubber tubing in a few minutes.



Part 3 Part 2 Part 1

Fig. 1.

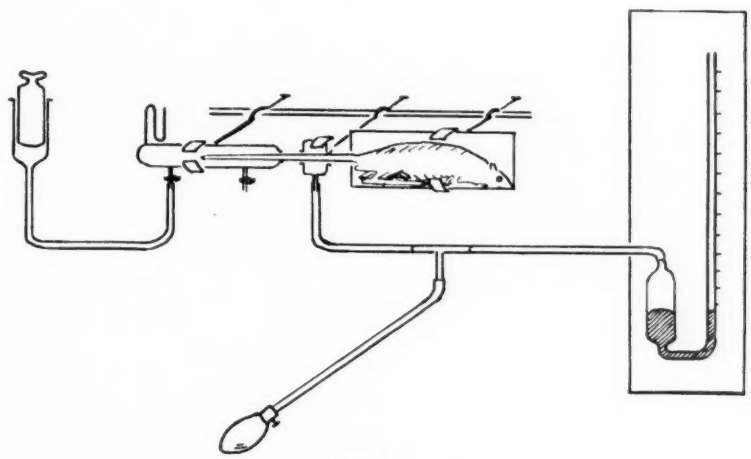


Fig. 2.

The general arrangement is shown in Fig. 2. The three parts are held together with clamps that slide on a rod. The pressure cuff is connected both to a mercury manometer and to a bulb with a shut-off valve, via a glass two-way connection. The water chamber is connected to a 50 c.c. syringe which acts as a water reservoir and allows the same water to be used without continually replenishing a simple water reservoir. The entire apparatus may be conveniently housed in a three-sided box.

The rat is gently placed in the holder that is then closed with the covering sleeve, allowing the entire length of the tail to protrude freely. The holder is placed in the clamp and the animal is allowed to sit for a minute to accustom itself to the holder. If the animal is very restless, an electric light placed beside the holder will usually give it a little warmth and quiet it. The tail

is then passed through the pressure cuff which is moved, together with its clamp, as close to the holder as possible. Soft vaseline is smeared on the distal end of the tail for about one inch from the cuff, and the tail passed through the sleeve into the water chamber. This latter is moved as close to the cuff as possible, so that a maximum of tail will be immersed in water. However, care must be taken that although the rubber sleeve is tight enough to prevent water leaking out around the tail, it is not tight enough to occlude the blood supply to the distal part of the tail. Such an adjustment is in practice quite simple.

Pressure is applied to the tail to a level of 30 to 50 mm. Hg above the expected systolic level. Water is run in from the syringe via the stopcock, filling the water chamber completely, and rising until the manometer is about half filled. Adjustment of the level in the manometer is most easily carried out by withdrawing water through the outlet stopcock. Pressure is then gradually released.

At systolic blood pressure blood will flow into the tail, and—being unable to return through the still occluded venous channels—will cause the tail to expand and the water to rise in the manometer. This expansion will be represented in the manometer by a rise of 2 cm. to 12 cm. or more. With practice it is possible to distinguish accurately two rates of expansion. The first occurs just at systolic pressure and is very slow so that the water level rises in small jerks. The second occurs 15 to 20 mm. below this and is rapid and fairly smooth. The first readings the beginner will make is this lower one, since it is more obvious, but with a little care the higher first point will be distinguished. It is our impression that the lower point corresponds to the unobstructed inflow of blood at diastolic, and hence that our two points probably correspond to those described by Diaz and Levy.

The following difficulties may be encountered. The animal, although not necessarily excited, may move its tail about. Such tail movements are readily distinguished from the true expansion by their jerky character, the large rise of water in the manometer, and the quick fall back again. If a tail movement occurs at an inappropriate moment, pressure is merely released and re-applied a second time. This may be repeated until one is certain of the reading. Another point of caution is that the water chamber should not contain any air, although one or two small bubbles will not interfere with the recording.

The method requires some practice but will, with care, give reliable and consistent results, both for single or repeated pressures, and requires very little time.

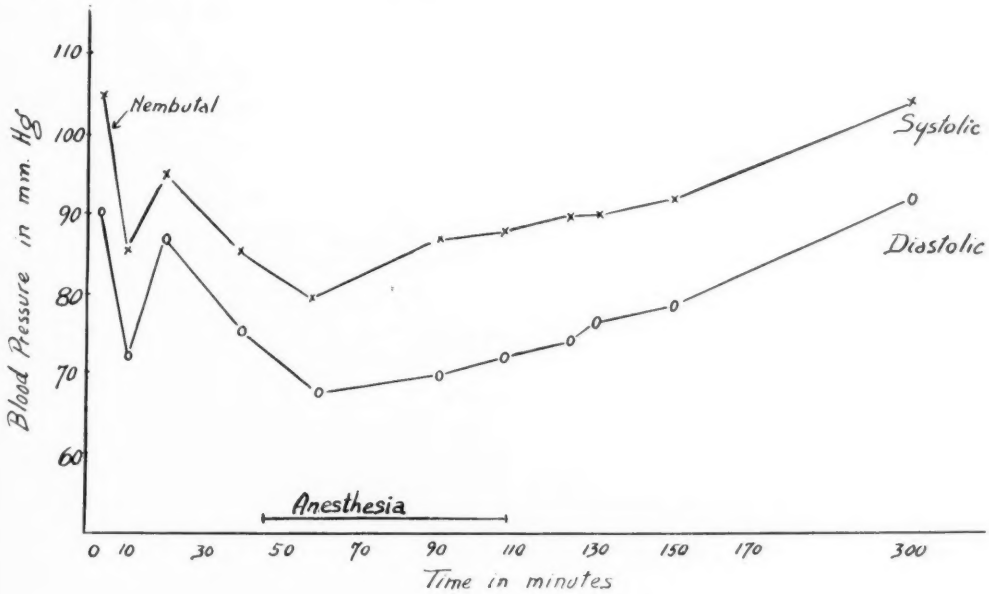
TABLE I

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12
Body weight	108	129	112	130	116	110	129	109	118	112	128	102
Systolic B.P.	104	108	108	108	106	110	110	108	110	108	104	104

We record in Table I the systolic pressure obtained on 12 normal rats with an average body weight of 124 Gm. The consistency of the pressure levels in these animals is striking and agrees well with the results obtained by direct manometry.[4]

In Graph I we record as an example the continuous systolic and diastolic blood pressures of a rat given a small dose of nembutal (sodium pentobarbital) for other purposes.

The normal pressure of the animal was first obtained, the injection was given, and within five minutes the animal was back in the holder and remained there until completely recovered. We wish to draw attention to the fact that more than half the points on the graph were plotted without the animal being under anesthesia, and to observe that diastolic readings represent the second point discussed in the foregoing description.



Graph 1.

SUMMARY AND CONCLUSIONS

1. A method for the determination of both systolic and diastolic pressures in the nonanesthetized rat is described.

2. Sample records are given to show the magnitude of the results obtained.

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AN AUTOMATIC QUADRUPLE PIPETTING MACHINE FOR THE RAPID AND ACCURATE DELIVERY OF MEASURED SMALL AMOUNTS OF FLUID*

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A NUMBER of automatic pipettes have been constructed for the delivery of accurately measured quantities of fluids in such tests as the complement fixation test for syphilis. The assemblies of Lorenz[1] and Laird[2] share the common and significant disadvantage of variation, and therefore, inaccuracy in delivery of fluids due to actuation of the two-way valve by the flow of fluid through it. The automatic pipette of Hartman,[3] incorporating a mechanically operated two-way valve, is sound in principle and accurate in operation, but the design of its construction does not readily lend itself to adaptation in a test like the New York State quantitative complement fixation test[4] for syphilis.

The desirability of an apparatus to dispense accurately and rapidly various fluid reagents in amounts ranging from 0.05 c.c. to 0.2 c.c. in the New York State quantitative complement fixation test for syphilis led to the development of an automatic quadruple pipetting machine of high accuracy and precision. Simple in construction and operation, it demands little or no manipulative skill. Apart from this test, the instrument is of value in any routine method, chemical or serologic in character, requiring the repeated delivery of measured small amounts of different fluids. The prime advantages of its design and construction lie in the elimination of complex gearing, belting, and rheostats, thereby avoiding the frequent breakdowns and changes in speed of delivery accompanying their use.

The instrument (Fig. 1) is driven by a $\frac{1}{20}$ H. P., A. C., 110 volt-60 cycle General Electric small synchronous motor, rotating at an absolutely constant speed of 75 r.p.m. From actual experience it has been determined that the optimum working speed for the average technician is 70 to 90 r.p.m., corresponding to an identical number of deliveries of fluid reagent. Hence, adjustment of the speed of the motor is not necessary. The synchronous motor provides a constant speed and eliminates all electrical regulating devices.

DESCRIPTION OF APPARATUS (Fig. 1)

An adjustable eccentric (*a*) fastened securely and directly to the shaft of the motor converts the rotary motion into a reciprocating one through a Scotch yoke (*b*), held rigidly by side gibs (*c*) which can be adjusted for take-up due to their wear.

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This eccentric (*a*) moves the pistons (*d*) of the syringes (*e*) in an upward stroke, and at the same time compresses the springs (*f*) which are placed in such a position that the pistons are held firmly against the reciprocating bar (*g*).

Noncorrosive heads (*h*), enclosing double-check valves of a ball-seating fully automatic gravity type, are connected directly to the Luer-Lok syringes. It has been found that this is the most simple and efficient design for the purpose of preventing the passage of fluid in either direction until demanded by the reciprocating stroke of the plunger. The valve is a simple and time-proved device, consisting of a ball seat and a truly spherical ball. The ball seat is angular. The weight of the ball sits in its angularity with a very narrow rim surface, thereby eliminating almost completely any danger of accumulation of foreign matter in the flowing fluids which would interfere with the function of the valve. The two-way valve is actuated by the mechanical action of the syringe plunger upon it with almost complete independence of the flow of the fluid reagents.

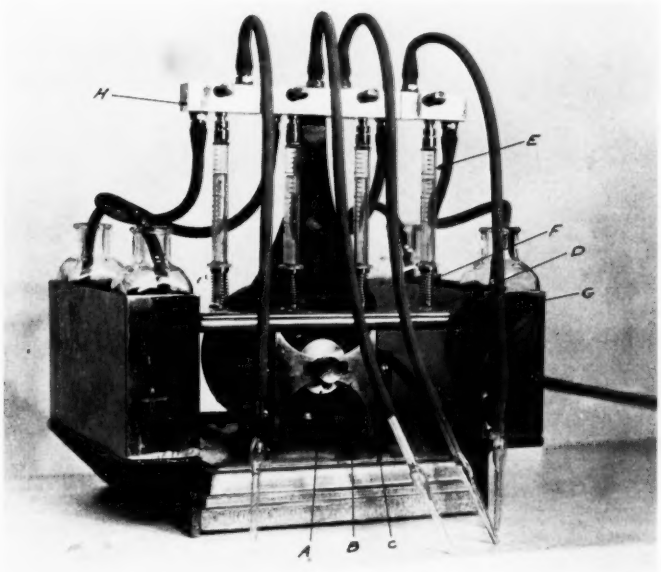

Fig. 1.

To the noncorrosive heads are attached inert rubber tubes of 2 mm. thickness in order to eliminate any pulsations leading to collapse of their walls. These, if present, represent a significant source of error in delivery.

Glass nozzles, prepared from carefully selected 1.0 c.c. standard Ostwald pipettes, are inserted into the outlet rubber tubes. It is manifest that each tube must deliver a constantly uniform volume of fluid without accumulation of a variably sized hanging drop about its opening during, or subsequent to, delivery. This was eliminated by altering the tip and opening the nozzle of the tube, and by polishing its end on a very fine oil stone, thereby eliminating the need of dipping it into oil. Failure to observe these precautions will lead to unequal and, therefore, inaccurate deliveries.

ACCURACY OF DELIVERY BY MACHINE

The accuracy of the pipetting machine was determined by weighing 10 successive deliveries of 1.0 c.c. water from each of four syringes. Table I shows the recorded weights of these deliveries at a constant temperature of 20° C., as determined on a Christian-Becker chain-o-matic balance. These figures indicate that the individual delivery from each pipette did not vary more than two parts per thousand, thus indicating the very high degree of each individual delivery with the pipetting machine. If even greater accuracy in the deliveries of all syringes is desired, four syringes of exact dimensions must be employed. These can be obtained only by selection from a large number of syringes by trial weighings of individual deliveries under standard conditions.

TABLE I

WEIGHT IN GRAMS OF SUCCESSIVE DELIVERIES OF SYRINGES

	SYRINGE 1	SYRINGE 2	SYRINGE 3	SYRINGE 4
	1.0048	1.0069	1.0024	0.9989
	1.0010	1.0025	1.0007	0.9973
	1.0012	1.0054	1.0024	0.9980
	1.0025	1.0042	1.0000	0.9965
	1.0045	1.0035	1.0035	0.9964
	1.0049	1.0025	1.0000	0.9971
	1.0018	1.0023	1.0011	1.0009
	1.0062	1.0050	1.0000	0.9998
	1.0050	1.0027	1.0015	1.0019
	1.0033	1.0029	1.0009	0.9988
Average weight	1.0035	1.0039	1.0012	0.9985
Mean deviation	0.00156	0.00129	0.00095	0.00150

Extensive experiments with pipetting by hand carried out under conditions identical with those for the test of the accuracy of the pipetting machine show that the maximum error is in the second significant place and lies between 0.9372 and 0.9982. This maximum percentage error of six parts per hundred, or 6 per cent, compares unfavorably with the maximum deviation of two parts per thousand, or 0.2 per cent, obtained with the automatic pipetting machine. This may be a significant source of error in that, with the pipetting of complement and antigen by hand in the New York State quantitative complement fixation test for syphilis, the possible cumulative error may rise to a total of 12 per cent. It is also of interest to note that all deliveries by hand were below 1.0 c.c., for which the pipettes were standardized. In the actual performance of the test, this would represent a second significant source of error in that smaller amounts of reagent are added than are required, thereby increasing the experimental error.

CARE OF THE MACHINES

The normal use of the automatic quadruple pipetting machine requires only a general lubrication of its moving parts when necessary, and flushing of the syringes and rubber tubing system with distilled water. The intake tube is immersed in distilled water, and by inserting a spatula beneath the plunger of the syringe and moving it up and down for about 20 full strokes, the

system is thoroughly flushed out. The intake tube is now removed from the distilled water, and with a few additional strokes of the plunger of the syringe, the system is emptied. The valve block need not be dissembled unless there is accumulation of foreign material on the valve seats, but this is extremely uncommon.

ADVANTAGES OF AUTOMATIC QUADRUPLE PIPETTING MACHINE

The advantages of the pipetting machine consist in the elimination of all adjustments after its first setting, increased speed and higher precision in the delivery of measured small amounts of fluid reagents, and absence of fatigue of the operator in comparison with pipetting by hand. In addition, there is a complete elimination of breakage and cleansing of serologic pipettes. The time required for the delivery of antigen and complement in various dilutions, salt solution, and sensitized sheep red blood cells in the New York State quantitative complement fixation test for syphilis is considerably reduced and accomplished with increased accuracy.

SUMMARY

An automatic quadruple pipetting machine for the accurate and rapid dispensing of liquids in amount of 0.05 c.c. to 0.2 c.c. in the New York State quantitative complement fixation test for syphilis is described. Its operation for over one year in our laboratory has given eminent satisfaction. The instrument can be readily adapted to other laboratory methods. For the delivery of larger amounts of fluid, larger syringes may be substituted.

The machine is manufactured by the Curtin-Hebert Co., Inc., Gloversville, N. Y.

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THE DETERMINATION OF SENSITIVITY TO PAIN

A Simple Clinical Method

Louis Pelner, M.D., Brooklyn, N. Y.

ALL who have read Emanuel Libman's[1] classical essay on the individual sensitiveness to pain must realize that without an appraisal of a patient's susceptibility to pain, there cannot be adequate diagnosis, prognosis, or treatment. The hyposensitive patient's complaints must be magnified in order to obtain a proper perspective. According to Libman,[1] a hyposensitive patient feels less pain, and the pain that he does feel has an unusual radiation. He may have substitution symptoms, e.g., pressure, burning, or numbness instead of pain. Every clinician can probably recall a case or two of a very acute surgical abdomen, where the surgeon was not consulted early because the patient did not have real pain, but had instead burning, pressure, or numbness over the abdomen. Libman believes that in the case of the hyposensitive patient, the pain impulses are delayed in the autonomic nervous system, while in the case of the hypersensitive patient, the impulse travels more directly to the central nervous system. He thus explains the occurrence of yawning, coughing, choking, and sneezing instead of pain in the hyposensitive patient.

There are many methods for measuring the intensity of the stimulus required to cause pain in the skin of a person. It is naturally assumed that the induced pain acts in the same way as a spontaneous pain. Roughly, the methods for inducing this stimulus are as follows: thermal, electrical, chemical, and mechanical. The thermal method was first used by Goldsheider in 1884, and has recently been made extremely accurate by Hardy, Woolf, and Goodell.[2] The drawback is the extensive equipment and the time necessary to determine the pain threshold of a person. It thus will have limited use except in a physiologic laboratory. The excellent papers of Hardy and co-workers show how well this method can be used on a small scale. The electrical method consists of stimulation of the skin with a faradic current and measuring the amount that will cause pain in a person. The exponents of this method who have done important work are Helmholtz, Martin,[3] Macht, Hauck and Neuert. The chemical method has been inadequately studied, possibly because stimulation by this method might cause an actual change in the skin. The mechanical method has been studied by von Frey, Eddy,[4] and others. Libman's test is an example of this method. In this test the examiner's thumb is pressed against the styloid process of the patient. This allows for a rough estimation of the person's sensitivity to pain. It can easily be seen that one factor in this test would be the strength of the examiner. There is, furthermore, an occasional difference between styloid processes. Recently Hollander[5] suggested an ingenious

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instrument that consists of a grater sewed to a blood pressure cuff. The cuff is applied in the usual way to the patient's arm and the cuff is inflated. The end point is reached when the patient cries out or winces. According to the most recent work,[6] this method is not considered accurate in obese or in moderately obese patients, and in those with severe or moderate hypertension. These restrictions limit the usefulness.

The instrument used in this study, the sensometer, is also a mechanical method of inducing pain in the skin. The one common drawback of the mechanical method is that the deformation and internal stresses of the skin and underlying tissues are not taken into account. In the method herein described, a part of the body is chosen that is not obese; it is felt that this reduces a serious drawback to the pressure method.

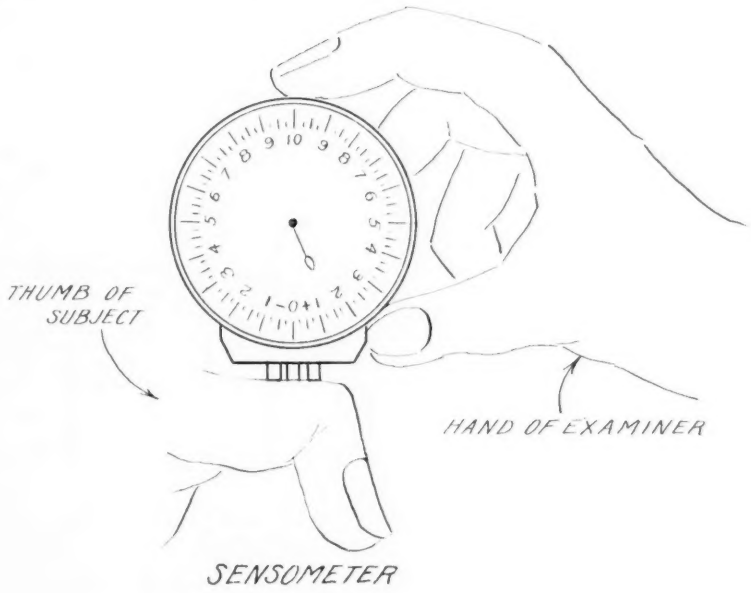

Fig. 1.

A NEW METHOD OF ESTIMATING PAIN SENSITIVITY

The device used in this study can be called a sensometer (Fig. 1). It is adapted easily from an instrument called the Geneva Lens Measure and is procurable as such all over the world. It consists of two peripheral fixed points, and one central point, which is attached to a hand on a watch dial. Originally the two fixed points were 2 cm. apart. These have been altered so that they are now 7 mm. apart. This can be done by the local watchmaker or jeweler. The reason for the alteration is that a flat surface is preferable for application of the stimulus, and it is difficult to find an area of the body that is flat for this distance (2 cm.). Also, too many nerve endings may be stimulated if the points are separated too widely; this may cause summation of impulses. However, readings are approximately the same with either measurement.

A relatively flat, nonfleshy portion of the body is chosen for this test. To insure uniformity in the survey, we have used the proximal phalanx of the

thumb held horizontally with the distal phalanx bent at right angles to it. The sensometer is rested on the phalanx, and the number that it registers of its own weight is kept in mind. Then the instrument is pressed into the skin until the pain becomes unbearable, and the number corresponding to this sensation is read. As one presses the two fixed points harder into the skin, the central point moves an equal distance, and this is measured on the dial. The difference between the figure read before and after pressure on the skin is taken as the patient's pain sensitivity. This instrument is standardized the same way all over the world. It can be tested before using by putting it on a perfectly flat surface; the watch hand will then read zero. In many months of constant use there has been no need for repair of the instrument.

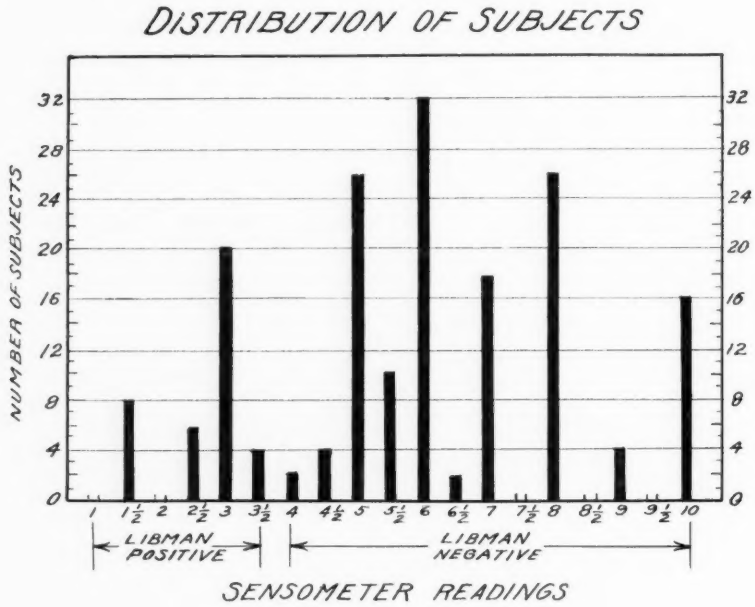

Fig. 2.

FINDINGS

A total of 178 patients and colleagues were tested repeatedly with the sensometer according to the method described. Each patient was also tested by the Libman method for comparison. The distribution of the results is indicated in Fig. 2. Hypersensitive patients represent about 22 per cent of the total tested, which agrees with the Libman figure of 30 per cent. Except for several minor variations, the Libman test agrees rather well with this study. The majority of the cases that were Libman positive (hypersensitive), ran from 0 to 3.5 units on the sensometer. The Libman negative cases (hyposensitive), with few exceptions, were over 3.5 units on the sensometer. It is in determining the degree of hypersensitivity or hyposensitivity that we feel that this method is superior to the Libman test.

There were 18 patients who deserve separate mention. Eight were positive to the Libman test on one side and negative on the other. Three of these, in spite of being hyposensitive, had had otitis media in their youth, which may account for a tender styloid process. Ten were unusual in one respect, al-

though the agreement between the sensometer readings and the Libman test matched closely. These were neurasthenic individuals, where one would expect a low score and highly positive Libman test, but where the reverse was true. These patients were Libman negative and had a high sensometer rating, but in each the feeling of pain induced by the stimulus lasted from twenty to thirty minutes to several days. Other neurasthenic individuals had low sensometer readings and highly positive Libman tests, but here also the sensation was prolonged. Normally, the feeling of pain caused by the pressure stimulus in either test lasted at most only two to three minutes.

One might postulate a theory from these facts as to the exaggeration of symptoms in certain neurasthenic individuals. There must be constant reinforcement of the stimulus in a neurasthenic individual, perhaps something in the nature of recurring afterimages. This may be caused by lack of certain substances in the body. Wilder[7] has recently restated a well-known fact that pellagrins cannot stand much pain; they feel this pain, however slight, for a long time, its disappearance coming only after adequate nicotinic acid therapy. This suggestive lead is given for research in the pain sensation of some neurasthenic individuals.

Eight patients were hypothyroid individuals. The basal metabolic rates ranged from minus 25 per cent to minus 9 per cent. The sensometer readings were high, some of the highest being in this class. The Libman test agreed very accurately with these readings. Whether these readings were actually high, or whether they were due to a slower perception and slower reaction time, merits separate investigation.

SUMMARY

1. A method of estimating the individual's susceptibility to pain is described.

2. This method is compared to the Libman test and a fairly close correlation is reached.

3. Pain sensitiveness in neurasthenic and hypothyroid patients is briefly analyzed.

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A TUBE FOR THE CENTRIFUGATION OF SIXTEEN SPECIMENS IN A FOUR-PLACE CENTRIFUGE*

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IN LABORATORIES in small hospitals serving large normal populations, the so-called "clinical centrifuge" is adequate for all ordinary purposes, but it may become the "bottle neck" in laboratory procedure in unusual circumstances, prolonging the day's work far beyond the time required by the other operations in the methods, and sometimes resulting in loss of unstable constituents. In a recent survey of vitamin C levels in the plasma of children in an orphanage, the "10 mm. centrifuge tube" shown in Fig. 1 eliminated this difficulty.

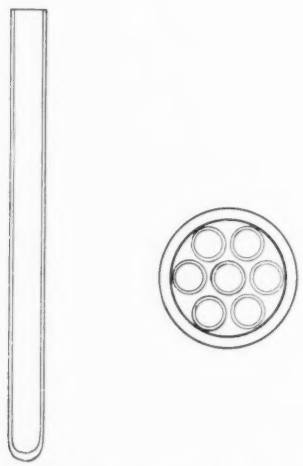

Fig. 1.—A 10 mm. centrifuge tube (left) and the arrangement of seven tubes in a 50 ml. brass centrifuge cup (right).

The International clinical centrifuge accommodates a four-place head having two 15 ml. and two 50 ml. brass cups, which can be purchased separately if the machine is not already equipped with it. One 10 mm. centrifuge tube can be placed in each 15 ml. cup, and seven 10 mm. tubes in each 50 ml. cup. Thus sixteen specimens may be centrifuged at one time. (If flat rubber pads are not at hand for the bottoms of the 50 ml. cups, they can be made by cutting a section from the middle of a No. 6 rubber stopper.)

Although the 10 mm. centrifuge tube was designed for use with the clinical centrifuge having a four-place head, it offers several advantages when used with the larger centrifuge and the eight-place head. Fourteen tubes containing specimens can be balanced in one operation instead of in seven. The depth

*From the Children's Hospital Research Foundation, and the Department of Pediatrics, College of Medicine, University of Cincinnati, Cincinnati.

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of the supernatant fluid makes it easier to draw off plasma without disturbing the cells. Exposure of the specimen to air is less, whether stoppered or unstoppered. The tube accommodates 4 ml., which is sufficient volume of specimen for many determinations for which blood is taken. It is sufficiently cheap and durable to be used for collection of the specimen, and the volume of specimen is easily estimated (approximately 1 ml. per inch).

The tubing selected for these centrifuge tubes must be between 9.5 and 10.0 mm., *preferably between 9.6 and 9.8 mm. in outside diameter.* The larger limit is the maximum which will permit the tubes to be easily placed in the 50 ml. cups, and the smaller limit insures room enough for inserting the tip of a transfer pipette or a 1 ml. graduated pipette. One end is sealed off as shown, and the tube is cut to a length of 110 mm. and fire-polished. (120 mm. is permissible for use with the larger centrifuge heads.) The end should be smoothly rounded and thick for mechanical strength. Tapered ends similar to those of the ordinary 15 ml. centrifuge tube were tried, but this offered no advantage and was even detrimental in operations in which the precipitate was to be resuspended, such as the washing of cells.

THE LAKING OF GLYCERINATED RED BLOOD CELLS BY PHYSIOLOGIC SALT SOLUTION*

HAROLD H. MITCHELL, B.S., M.S., ST. LOUIS, MO.

PERFORMING the Wassermann reaction with high titer amboceptor serum, containing 50 per cent glycerin as a preservative, presents no complications. However, with an amboceptor serum of low titer we encountered a complication which was due to the contained glycerin. After the red blood corpuscles had remained in contact with the relatively large amount of amboceptor serum (necessitated by its low titer) for an hour or longer, they were subject to immediate lysis when added to 0.85 per cent salt solution.

Inquiry revealed that this phenomenon was due to the slow diffusion of glycerin into the red blood corpuscles. The osmotic pressure developed upon introduction into the glycerol-free normal salt solution caused sudden lysis of cells. This degree of osmotic change occurs if the amount of amboceptor necessary to sensitize a given volume of cells results in addition of such an amount of glycerin that its final concentration in the cell suspensions reaches 2 per cent or more.

The glycerinated cells could be brought back to normal stability by washing in hypertonic salt solution or by addition of normal salt solution very slowly in divided amounts.

It is suggested that low titer amboceptor be preserved by some means other than glycerin, or that cells subjected to a large amount of glycerin be suitably washed before use.

*From the Department of Bacteriology, Washington University School of Medicine, St. Louis.

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A RAPID METHOD OF DEHYDRATING AND CLEARING IRON-HEMATOXYLIN STAINED FECAL SMEARS*

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THE preparation of wet-fixed stained smears for the identification of intestinal protozoa is a time-consuming procedure and is, therefore, considered by many impractical for clinical laboratories. Any modification which shortens the methods used should be of practical interest.

I have found that a preparation called cellosolve (ethylene glycol monoethyl ether) can be used for dehydration and clearing instead of alcohol and xylol, and thereby a considerable amount of time can be saved. Instead of the eight changes usually used for the dehydration and clearing with alcohol and xylol, only a single immersion of ten minutes in cellosolve is required.

The use of cellosolve also has the advantage of being less expensive. A dehydration-clearing set-up of alcohol and xylol (eight dishes) costs about $1.30, while the cost of a dish of cellosolve for the same purpose is less than 20 cents. Furthermore, the cellosolve can be used longer and for a greater number of smears before losing its efficacy.

By using a short method of staining, as suggested by Johnson,[1] the whole procedure of fixing, staining, dehydration, and clearing can be accomplished in about an hour.

The timing of the various steps in the method now used at the United States Naval Medical School is as follows:

Fix in Schaudinn's solution (glacial acetic acid to strength of 5 per cent added)	5 minutes.
Alcohol, 95 per cent, with iodine to a port wine color	3 minutes.
Alcohol, 70 per cent	3 minutes.
Rinse in tap water.	
Ferric ammonium sulfate, 4 per cent	15 minutes.
Rinse in tap water.	
Hematoxylin, aqueous solution, 0.5 per cent	10 minutes.
Ferric ammonium sulfate, 0.25 per cent	12 minutes.†
Wash in running water	1 minute.
Cellosolve	10 minutes.
Mount in balsam.	

The foregoing method has now been used for a year and has been found entirely satisfactory in the routine work of identifying the intestinal protozoa. As far as can be determined at this time the stains are as permanent as those dehydrated and cleared in alcohol and xylol.

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*From the United States Naval Medical School, Washington, D. C.

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†Varies somewhat with each batch of hematoxylin. The time may be determined by taking slides through at periods of eleven, twelve, and thirteen minutes, and noting the best result.

AN IMPROVED METHOD FOR THE ESTIMATION OF QUANTITATIVE AND QUALITATIVE PLATELET FACTORS*

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CHICAGO, ILL.

IN 1936 we described a method for the quantitative and qualitative estimations of platelets in their own plasma.[1] During the past five years we have shortened and simplified this method until we now believe that it may be easily and simply done in any laboratory.

METHOD

No anticoagulants are used since such substances mask the extent to which platelets may be broken down in the original blood sample and so interfere with the correct judgment of results. This test requires 1 c.c. of blood, withdrawn from a vein into an iced, oiled syringe, and introduced into a paraffined tube imbedded in ice. We have found that the graduated 1 c.c. tubes used for sedimentation time are excellent for this purpose. These tubes are paraffined with a mixture of two-thirds paraffin and one-third mineral oil. The tubes are then packed in ice in a large centrifuge cup and centrifuged for one and one-half minutes at 5,000 r.p.m..

After one and one-half minutes centrifuging the platelet count is made as follows: A few grains of heparin are placed on a watch crystal which has been thoroughly chilled, and resting in ice. A sample from the center of the centrifuged plasma is drawn up into a capillary tube, and several drops are mixed with the heparin. Following thorough mixing, a drop of this mixture is placed in a chilled counting chamber, covered with a chilled cover glass, and placed in a refrigerator or under ice for fifteen minutes to allow for settling. The platelets in 80 small squares are then counted, and the results are multiplied by 50 to obtain the number of platelets in a cubic millimeter (the platelets in the 80 small squares represent $\frac{1}{50}$ c.mm.). The platelet counts obtained by the method described must be considered as plasma platelet counts and should be further corrected for whole blood. This is obtained by the hematocrit reading obtained later. The plasma platelet count multiplied by the plasma percentage gives the platelet count for the whole blood.

At the same time that the plasma is placed on the watch glass for the platelet count, the remainder of the plasma left in the capillary tube is used to estimate the coagulation time of the plasma. This is done by breaking off pieces of the tube, as in the method of Rudolph, until a thread of plasma has formed. This represents the coagulation time of the platelet containing plasma,

*From the Department of Pediatrics, Rush Medical College, and the Presbyterian Hospital, Chicago.

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and is designated plasma A. As soon as the capillary tube of plasma is withdrawn from the original paraffined tube containing the blood sample, the latter is again placed in ice and centrifuged for fifteen minutes. At the end of this time another sample of the centrifuged plasma, which is now platelet free, is taken into a capillary pipette and the coagulation time is obtained as before. This plasma is designated plasma B. The hematocrit reading may now be made for calculation of the total platelets in the blood sample.

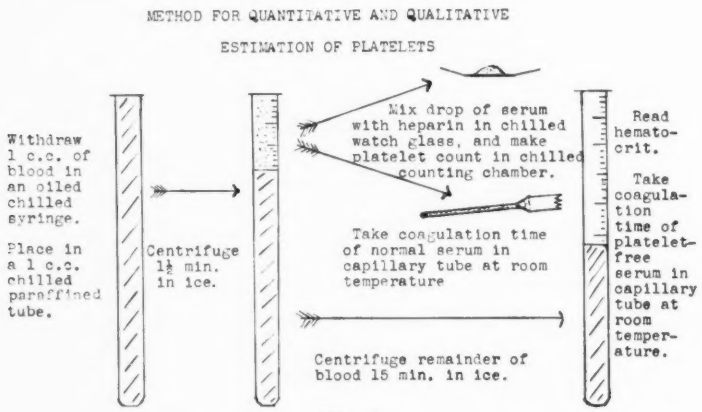
METHOD FOR QUANTITATIVE AND QUALITATIVE
ESTIMATION OF PLATELETS
Withdraw 1 c.c. of blood in an oiled chilled syringe.
Place in a 1 c.c. chilled paraffined tube.
Centrifuge 1½ min. in ice.
Mix drop of serum with heparin in chilled watch glass, and make platelet count in chilled counting chamber.
Take coagulation time of normal serum in capillary tube at room temperature
Centrifuge remainder of blood 15 min. in ice.
Read hematocrit.
Take coagulation time of platelet-free serum in capillary tube at room temperature.

Fig. 1.

The principal changes from our original method reported in 1936 are the elimination of all time-consuming processes dealing with plasma fractions which we do not believe are clinically necessary. Also in shortening the centrifuging of the plasma for fifteen minutes instead of one and one-half hours as formerly used, we have reduced the time so that the entire estimations may be done in twenty minutes. It must be understood that values for plasma B depend on the length of time taken in centrifuging. The longer the plasma is centrifuged, the more platelets will be thrown down, and conversely, the longer will be the coagulation time of this plasma. Fifteen minutes was taken as the time for this centrifuging because it is the shortest time in which all microscopically visible platelets can be centrifuged from the plasma. It would be well before undertaking this test for each worker to test his individual centrifuge by observing if there were any platelets in the plasma after fifteen minutes of centrifuging.

With this procedure we have for consideration a plasma platelet count, the coagulation time of natural plasma with its full number of platelets, and the coagulation time of platelet-free plasma. It must be emphasized that if any difficulty is encountered in obtaining the blood so that there is the slightest addition of tissue fluid or cerebrospinal fluid, the results are unreliable. This can be immediately detected by the platelet count. It will be lower than anticipated, and the platelets will show clumping and variation in size. Any platelet count of less than 100,000 should be viewed with suspicion and repeated. If the procedure is carried out under these conditions, one of two things will occur. First, all the coagulation times will be abnormally short, or plasma B may even coagulate in the centrifuge tube. This should be suspected if plasma A coagulates in less than four minutes. Secondly, in the opposite extreme all

coagulation times may be abnormally long, and plasma B may never coagulate because coagulation has occurred and the supernatant fluid is serum rather than plasma. This is noted especially if cerebrospinal fluid is allowed to get into the sample. This difficulty would probably not occur in obtaining blood from adults, but in pediatric work in newborn and small infants blood is frequently withdrawn from the longitudinal sinus, where cerebrospinal fluid is very easily drawn in with the blood. It is very important, therefore, to know if there have been such additions to the blood. This method of checking is far from perfect, but when anticoagulants are used, no check at all is possible.

RESULTS

Normal adults show a plasma platelet count varying between 100,000 and 400,000 per c.mm. The coagulation time for the normal plasma (plasma A) is between four and eight minutes, and for platelet-free plasma (plasma B) between six and ten minutes.

The first deviation from these standards is shown in individuals with hemorrhagic symptoms, but with a normal or higher platelet count. We have found this in the following conditions:

1. Hemophilia. This condition will show values for plasma A and plasma B of from ten to twenty times the normal value. Following transfusion, plasma A will drop to long normal values, but plasma B will still remain long, even during periods that are hemorrhage free. In one instance in a child, aged 1 year, in which the maternal uncle has hemophilia, the platelets were 360,000 with a coagulation time of twenty-four minutes for plasma A and twenty-seven minutes for plasma B. This boy has had some petechial hemorrhages and large ecchymotic areas after small bruises, but no gross hemorrhages have occurred. We feel that the diagnosis of hemophilia is justified in this instance.

2. Pathology of the liver and gall bladder, or bleeding with jaundice. This can be divided into two groups:

a. In which a deficiency of prothrombin also occurs. In these instances both plasma A and plasma B will be somewhat prolonged in proportion to the level of prothrombin. Our ratio has been as follows:

Prothrombin 30 per cent, plasma A 10, plasma B 11
Prothrombin 40 per cent, plasma A 9, plasma B 11
Prothrombin 50 per cent, plasma A 8, plasma B 10
Prothrombin 60 per cent, plasma A 6, plasma B 7
Prothrombin 70 per cent, plasma A 6, plasma B 6

b. In which the prothrombin is of normal value. In several instances we have found an increase in both plasma A and plasma B values with relatively normal prothrombin. For example, in a carcinoma of the gall bladder region, the coagulation time for plasma A was twelve minutes, and that for plasma B was fourteen minutes; the prothrombin value was 75 per cent.

3. A very small group, which except in one instance was characterized by intestinal bleeding, is that in which the platelets are normal or even higher than normal with slightly increased values for plasma A but considerable increase for plasma B. The prothrombin was normal in all instances. With the one exception of an endocarditis with petechial hemorrhages, the remaining four in this group had intestinal bleeding. Three were considered to have

ulcerative colitis. The fourth was a boy whom we have followed for several years, whose case has been diagnosed as Henoch's purpura. In one instance when he was seen immediately after a very severe bleeding from the bowel, his blood showed 325,000 platelets, his plasma A had a coagulation time of nine minutes, and his plasma B eighteen minutes. Since all these patients had normal platelet counts, the delayed coagulation time would seem necessarily due to an increased platelet resistance.

The second and by far the larger group deviating from normal is that in which the platelets are less than normal. We consider this to be the case when the platelets are less than 100,000 by this method.

1. This group includes those with anemias, leucemias, and infections that affect the blood stream. These are all characterized by a thrombocytopenia, proportional increase of coagulation time in both plasmas A and B, and a low hematocrit. We felt that one should never make a diagnosis of primary thrombocytopenia with a low hematocrit. It is almost certain to be of a secondary type. Examples of these are aplastic anemia: Platelets 24,000; plasma A coagulation time nine minutes; plasma B, fifteen minutes; hematocrit 10 per cent; marked hemorrhage. Leucemia: Platelets 124,000, plasma A coagulation time seven minutes; plasma B, eight minutes; hematocrit 40 per cent; no bleeding. Same child when platelets were 23,000: plasma A coagulation time eight minutes; plasma B thirteen minutes; hematocrit 25 per cent: marked hemorrhages. Infected mastoid (streptococcus): platelets 40,000, plasma A coagulation time eight minutes; plasma B, fifteen minutes; hematocrit 30 per cent: ecchymosis and petechia. After transfusion the platelets were 180,000, plasma A coagulation time was five minutes, plasma B was seven minutes, and hematocrit was 40 per cent.

The findings in these cases of thrombocytopenia suggest that in this group we are dealing with platelets which, though reduced in number, are fairly normal in character. They undergo relatively little disintegration during the process of centrifuging them out of the plasma, and the coagulation time of plasma A is prolonged in most cases, proportionally to the reduction in total platelet count.

2. Thrombocytopenia, with increased coagulation time of plasmas A and B, but with these practically equal in time. The majority of these individuals have had petechiae and hemorrhages in different parts of the body over a long period of time. A ten-year-old girl had petechial hemorrhages, and bleeding from the mucous membranes for five years: platelets 5,000; plasma A coagulation time eighteen minutes; plasma B, eighteen minutes. A 13-year-old boy had similar symptoms and bleeding over a period of three years, with several transfusions: platelets 4,000; plasma A coagulation time, twenty minutes; plasma B, twenty-two minutes. Following transfusions with increases in the platelet count, plasma A will shorten to almost normal, while plasma B will remain at double the normal coagulation time.

In this group we are dealing with longer standing hemorrhagic disturbances, resembling the syndrome described by Werlhof. Here the platelets do not show a normal resistance but are broken down during centrifugation, yielding a platelet-free plasma which coagulates as rapidly as the normal plasma (plasma A).

3. Thrombocytopenia, with long normal coagulation time, and only occasional hemorrhagic symptoms. Persons of this group have occasional petechiae and small areas of ecchymosis, with some very slight bleeding of the mucous membrances. Their platelets vary from 5,000 to 50,000; their plasma A coagulation time is from four to ten minutes, and their plasma B, from nine to eleven minutes. Their hematocrit, prothrombin, and vitamin C blood content are all normal. We have called such instances "congenital thrombocytopenia," and described a family in such a state in 1936.[2] Since that time we have found twelve more such patients, but have been unable to trace any family connection as in the first family. It will be noted that while their normal plasma coagulates normally, their platelet-free plasma is a little longer than normal. This would indicate that their platelets are very active and that coagulation time is delayed only because of a failure in gross number. Such individuals do not have fatal hemorrhages because their platelets disintegrate more rapidly than do normal platelets. However, some factors might occur that would reduce or lessen this ability to disintegrate and cause a profuse hemorrhage. As long as no exceptional stress or strain is put on persons with this condition, they are able to have normal blood-clotting functions, but in the presence of an acute infection or debilitating condition, the increased power of disintegration of the platelets is lost and a profuse hemorrhage may occur.

One of the most interesting findings to us in this study is the fact that the gross quantity of the platelets in the blood does not seem to make a proportional difference in the coagulation ability of the blood or plasma. Platelet counts of 100,000 to 400,000 will all show approximately the same coagulation values. Even if the platelet value is reduced below 100,000 there is not a great deal of change until the number is decreased below 30,000. We have found individuals with platelet counts of 5,000 and only slightly increased coagulation values. On the other extreme we have hemophilias with platelet values of 500,000, with practically no blood coagulation at all. We have come to pay very little attention to the gross quantitative platelet value, but use it simply as a check on our technique. As stated before, the admission of even the smallest quantity of tissue fluid or cerebrospinal fluid to the blood will decrease the platelet number and give faulty coagulation values. A platelet value of less than 100,000 should be thoroughly rechecked. We believe, therefore, that the most important function of the platelets is not their number or quantity, but their activity or disintegration ability. This can be demonstrated by the coagulation of the plasma fractions as described.

SUMMARY

A clinical method for the estimation of platelets quantitatively and qualitatively in their own plasma, without the use of anticoagulants, is described.

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CHEMICAL

PEPTONE IN PROTEIN HYDROLYSATES AND AMINO ACID MIXTURES*

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INTRODUCTION

HYDROLYTIC reagents split protein materials into molecular units of various size. Such degradation products have been classified, supposedly in the order of their molecular weight, as protein, metaprotein, proteose, peptone, subpeptone, and amino acids. Classification is based in part on solubility behavior and reactions which certain hydrolysates display toward reagents.

The peptone fraction of hydrolysates has been the subject of much discussion. Products designated as "peptone" are widely used as a medium for bacterial growth, in jejunal feeding, and in some supposedly therapeutic diets. But even completely hydrolyzed proteins have been decried for parenteral use because of possible "peptone" shock. However, the factor responsible for the production of shock has never been fully established. Two classes of materials have been incriminated: peptone itself, and by-products resulting from the hydrolysis. It has been the common observation that Witte's peptone produces shock[1] or some phase of the shock phenomenon.[2, 3] Thus Henriques and Andersen[4] observed no shock from the parenteral injection of enzymatically hydrolyzed meat protein, yet the injection of Witte's peptone resulted in the death of his animals. Likewise, Milles and Seed[5] gave repeated injections of Difco's bacto-peptone to dogs and were unable to detect any toxic effects. It is thus evident that some peptones produce shock and others do not. It is quite possible that the method used to prepare peptone products is largely responsible for their shock-producing quality, and that "peptone" itself need not necessarily be involved.

It is difficult for the chemist to define what is meant by peptone. The term is a vague one, and though it has been commonly used for years, it does not seem to denote a chemical entity. The several proposals which have been made for its quantitative estimation involve its precipitation from aqueous solution. The earlier procedures utilized phosphotungstic acid as the precipitant, but these methods are now recognized as unreliable due to the partial precipitation of certain basic amino acids which are usually present in the solution. The use of bromine[6] as a precipitant for peptone was discarded at an early date when it was demonstrated that such treatment resulted in the loss of nitrogen. Gubareff and Sergejewa[7] utilized the biuret reaction for estimating peptone after the solution had been heated to coagulate unhydrolyzed protein.

*From the Laboratories of Mead Johnson & Co., Evansville, Ind.
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Such a procedure is unsatisfactory, since it will include all substances not coagulated by heat that give a positive biuret reaction. Schjerning[8] outlined a procedure utilizing tannic acid as the precipitant for the peptone fraction. Bigelow and Cook[9] improved his early technique and claimed that if a correction for creatinine (precipitated by tannic acid) was made, good results could be obtained. They found that a low temperature and a definite concentration of tannic acid and salt were necessary for maximum precipitation of peptone nitrogen. After a careful study of the factors affecting the precipitation of peptone by tannic acid, Wasteneys and Borsook[10] outlined a method for its estimation in enzymic digests of protein under carefully controlled conditions. This is the only available and dependable method for the determination of peptone, and in the experiments below we have followed their outlined procedure.

EXPERIMENTAL

An acid and an enzymic digest of casein, a mixture of crystalline amino acids, and Difco's bacto-peptone were studied for their peptone content.[10] The enzymic digest was prepared in a manner previously described.[11] The acid digest was prepared by boiling casein in 25 per cent sulfuric acid and then carefully neutralizing the sulfuric acid with barium hydroxide. The filtrate was decolorized and dried to a fine powder in an atmospheric spray dryer. The mixture of crystalline amino acids contained twenty of the twenty-three usually accepted amino acids in the amounts used by Shohl and Blackfan.[12] Hydroxyglutamic acid, thyroxine, and diiodotyrosin were omitted. Difco's bactopeptone, as purchased, was the fourth substance.

None of the four substances contained protein or proteose, since they gave no precipitate with trichloracetic acid or when the solution was saturated with sodium sulfate. The solution was prepared for the peptone determinations by dissolving 10.0 Gm. of the dry powder in 100 c.c. of 22 per cent sodium sulfate solution, adjusting the pH to 7.0 by the addition of a few drops of 60 per cent sodium hydroxide, and diluting to a volume of 200 c.c. with the sodium sulfate solution. To 25 c.c. aliquots were added an equal volume of 2.21 N sodium hydroxide and 125 c.c. of 20 per cent tannic acid dissolved in 0.1 N sulfuric acid containing 20 per cent sodium sulfate. The mixture was thoroughly shaken and kept at 20° C. for four hours. The solution was then filtered and nitrogen determinations were made on the filtrate. All determinations were run in triplicate by the Kjeldahl-Gunning method, and suitable correction made for the nitrogen content of the reagents. The difference between the initial nitrogen content of the original solution and the nitrogen content of the filtrate is expressed as the percentage of peptone nitrogen precipitated.

The results obtained are given in Table I. The apparent peptone content of the enzymic hydrolysate was 26.9 per cent, since this amount of the total nitrogen was precipitated by the tannic acid. The acid hydrolysate gave a figure closely approximating this, i.e., 22.73 per cent. Most surprisingly, the mixture of crystalline amino acids showed an apparent peptone content of 21.36 per cent. These average findings are all so closely similar that they raise the question as to whether all three preparations contain the same amount of peptone, or whether there is any peptone present at all.

It is evident that there is no peptone in a mixture of pure crystalline amino acids. Also, it is generally believed that prolonged boiling with strong acid reduces complex nitrogenous molecules almost entirely to the state of amino acids. At most, some dipeptide linkages may be present. We are, therefore, left with the finding that a mixture known to consist solely of amino acids, and a second mixture known to consist predominantly of such groups, but with possibly some dipeptide linkages, have essentially the same apparent peptone content of 21 to 23 per cent. The conclusion is obviously untenable. The observed differences, if they mean anything at all, do not represent anything in regard to peptone, and at most may represent differences in the amino acid content of the different substances employed.

TABLE I

PRECIPITATION OF NITROGEN FROM PROTEIN HYDROLYSATES AND AMINO ACID MIXTURE

SUBSTANCE	TOTAL NITROGEN			NITROGEN PRECIPITATED (%)
	IN ORIGINAL ALIQUOT (MG.)	IN FILTRATE (MG.)	NITROGEN PPT. (MG.)	
Enzymatically hydrolyzed casein	150	111	39	26.0
	150	108	42	28.0
	155	110	45	29.0
	155	109	46	29.7
	150	115	35	23.3
	147	111	36	24.5
	147	111	36	24.5
	153	104	49	32.0
	153	114	39	25.5
	153	112	41	26.8
	153	114	39	25.5
	151	111	40	26.5
	151	108	43	28.5
Average	–	–	–	26.90
Acid hydrolyzed casein	157	124	33	21.0
	160	121	39	24.4
	159	120	39	24.5
	157	123	34	21.7
	157	121	36	22.9
	157	125	32	20.4
	157	123	34	21.7
	157	120	37	23.6
	157	124	33	21.0
	157	116	41	26.1
Average	–	–	–	22.73
Mixture crystalline amino acids	134	105	29	21.6
	139	106	33	23.7
	136	107	29	21.3
	133	105	28	22.1
	133	102	31	23.3
	132	104	28	21.2
	132	106	26	19.7
	132	107	25	18.9
	132	105	27	20.5
Average	–	–	–	21.36
Bactopeptone	192	50	142	73.96
	184	47	137	74.46
Average	–	–	–	74.21

DISCUSSION

The report demonstrates the difficulty of arriving at any conclusion on the peptone content of the various materials. A mixture of crystalline amino acids, an acid hydrolysate of casein, and an enzymic digest of casein have been shown to have an apparent peptone content of 21, 23, and 27 per cent, respectively.

The differences in the apparent peptone content of the three widely different substances were not large and can be accounted for solely on the basis of the variability of the method itself. It thus seems impossible to determine what meaning may be attached to the finding that 74 per cent of the nitrogen of a preparation reportedly containing peptone is precipitated by tannic acid.

On the other hand, the precipitation of 74 per cent of the nitrogen of bacto-peptone indicates something. This larger amount of precipitated nitrogen cannot logically be explained on a basis of differing amino acid composition. It probably represents, in part, the presence of some larger molecular units. It seems quite possible, however, that it may not all be representative of such larger units. Nor would it seem advisable to use the figure 21.4 obtained for the mixture of crystalline amino acids as kind of blank for other approximations of peptone, since it can be interpreted from our findings that the amount of nitrogen precipitated by tannic acid depends to some extent on the percentage composition of the particular amino acid mixture used. The mixture of Shohl and Blackfan[12] may or may not approximately represent the composition of the digests, even though their mixture was designed to simulate the composition of casein. The acid digest which we employed contained no tryptophan, and certain portions of other amino acids may have been lost by adsorption on the precipitated barium sulfate. The enzymic digest, although probably containing most of the casein nitrogen, contained in addition a certain amount of nitrogen from the pancreas. We are thus left in doubt as to the significance of the concept of peptone.

SUMMARY

Precipitation with tannic acid is the only currently employed method for the estimation of peptone. The procedure results in the precipitation of 21 per cent of the nitrogen of a mixture of crystalline amino acids, 23 per cent of the nitrogen of an acid hydrolyzed protein, 27 per cent of the nitrogen of an enzymic digest of protein, and 74 per cent of the nitrogen of bacto-peptone. The method cannot be regarded as at all definitive for peptone.

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SIMPLIFIED CALCULATION AND NOMOGRAM OF THE VAN SLYKE UREA CLEARANCE TEST*

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DURING the last decades there has been an increasing interest in the functional capacities of diseased organs. This has given rise to various functional tests. One of the most important of these has been the Van Slyke urea clearance test for renal function. This test has almost generally been accepted as most reliable and has become a routine test in many hospitals. Van Slyke's formula is:

$$\frac{U}{B}\sqrt{C}$$

where U = urea content of urine, B = same of blood, C = urine output in one minute. C is calculated by dividing the output for one hour, H, by 60. The result is expressed in per cents of 54, this being the average value in healthy people. The actual calculation, therefore, is:

$$\frac{U}{B}\sqrt{\frac{H}{60}} \times \frac{100}{54}.$$

To reduce the daily loss of time in calculating this formula we used the transposition:

$$\frac{U}{B}\sqrt{\frac{H}{60}} \times \frac{100}{54} = \frac{U}{B}\sqrt{H} \times \frac{100}{54\sqrt{60}} = \frac{U}{B}\sqrt{H} \times 0.2391$$

$$= \frac{\sqrt{H}}{B} \times 0.2391\ U = \frac{\sqrt{H}}{B} \times 0.24\ U.$$

Substituting 0.24 for 0.2391 gives an error of only 0.4 per cent, negligible in routine work. This can be read on a slide rule in a few seconds. However, since medical men in general are not acquainted with the use of a slide rule, we have drawn up a nomogram by transposing the formula to read thus:

$$\frac{U}{B} \times 0.2391\ \sqrt{H}.$$

To read the nomogram the figure representing the urea content of the urine and the figure representing the blood urea are connected by a straight line. (A

*From the Paramaribo Medical School, Dutch Guiana.
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black thread is most suitable.) The point of intersection of this line and the U/B line is connected with the figure representing the urine output in one hour. The point of intersection of this second line and the scale gives the product of the formula in per cent.

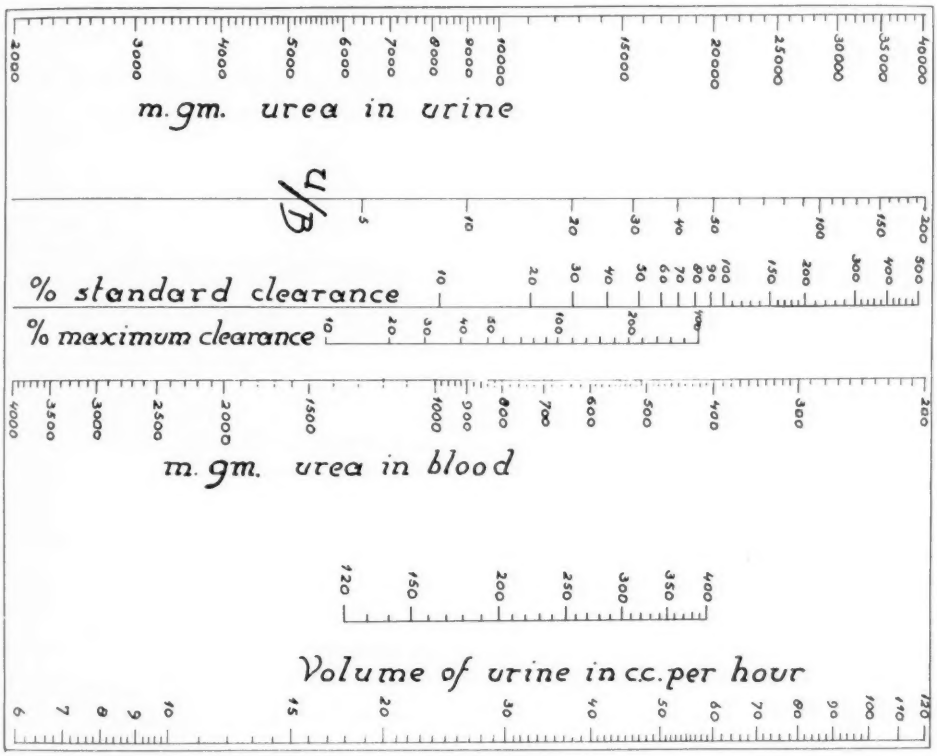

Fig. 1.

A scale for the maximum clearance has been worked out on the same principle. For instance:

U = 20,000, B = 1,000, urine per hour 70 c.c.

A line from 20,000 to 1,000 crosses U/B at 20. Connect 20 with 70 (cubic centimeters per hour). This line intersects the standard clearance scale at 40; that is, the standard clearance is 40 per cent.

In our medical ward the nomogram has been in use for nearly a year and has proved to be efficient and very simple.

A NOTE ON THE USE OF HENGAR SELENIZED GRANULES IN THE DIGESTION PROCEDURE FOR THE MICROGASOMETRIC DETERMINATION OF NITROGEN*

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THE use of potassium persulfate as catalyst and oxidizing agent in the digestion mixture as recommended by Wong,[1] and adapted by Peters and Van Slyke,[2] fails to give consistent results. The destruction of the excess persulfate by the repeated addition of water to the digest cannot be effected uniformly. As a result the oxygen liberated by the reaction of the hypobromite and the residual persulfate in the gas chamber of the Van Slyke-Neill apparatus gives high values. The excess persulfate can be completely destroyed by the addition of oxalic acid, but this is a complicating and time-consuming procedure.

The use of a single Hengar selenized granule in 2 c.c. of a mixture consisting of three volumes of sulfuric acid (sp. gr. 1.84) and one volume of phosphoric acid (sp. gr. 1.71) insures complete digestion in ten to fifteen minutes. Not only is the oxidation catalyzed by the selenium, but bumping is eliminated by the porous granule. This digest is neutralized and treated with hypobromite in the manometric apparatus, as outlined by Peters and Van Slyke,[3] with results which check in duplicate, as indicated in Table I.

TABLE I*

NITROGEN CONTENT OF PLASMA

SAMPLE	VOLUME TAKEN	MG. NITROGEN PER C.C.
1	0.1 c.c.	11.88, 11.80
2	0.1 c.c.	12.06, 12.22
3	0.1 c.c.	12.28, 12.10
4	0.1 c.c.	12.03, 12.08
5	0.1 c.c.	11.58, 11.51

*The results given here are duplicate values on consecutive samples taken in routine experiments.

By this procedure quantities of nitrogen from 0.5 to 1.5 mg. can be determined with an expected accuracy of one-half of 1 per cent. The blank corrections are consistently low.

SUMMARY

A modified procedure is presented for the preparation of the digest in the microgasometric determination of nitrogen.

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3. Loc. cit., page 357.

Hengar selenized granules may be obtained from the Hengar Company, Philadelphia, Pa.

*From the Surgical Hunterian Laboratory, Department of Surgery, the Johns Hopkins School of Medicine, Baltimore.

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A MODIFICATION OF THE PUCHER-SHERMAN-VICKERY METHOD FOR THE DETERMINATION OF CITRIC ACID*

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THE concentration of citric acid in blood or serum has been a subject of much interest since Sjöström[1] found that this concentration was increased in cases of injuries of the parenchyma of the liver. According to the experience of this laboratory, the test is one of the best laboratory methods for the differentiation of catarrhal jaundice (acute hepatitis) and icterus due to obstruction of the bile ducts. The test does not seem to be so sensitive as the hippuric acid test, though it is perhaps more specific than the latter.

Sjöström and several later investigators used Thunberg's method. In this method the citric acid decolorizes the dye methylene blue by means of an enzyme, citric acid dehydrogenase, from cucumber seeds. The concentration is calculated from the time needed for a certain amount of the serum to decolorize a certain amount of the dye. The method is rather time-consuming, and the decolorization times are not easy to establish with accuracy.

The chemical citric acid method by Pucher, Sherman, and Vickery[2] (the penta-bromacetone method) thus represented a definite advantage. In this method the citric acid is turned to pentabromoacetone, giving a yellow-brown color with sodium sulfide proportional to the amount of acid. The method has been much used in Sweden and other Scandinavian laboratories, and has proved to be satisfactory.

This method has, however, one drawback. The color developed is rather unstable and it fades in such short time that it can scarcely be used for colorimetry. For this reason, Pucher, Sherman, and Vickery mixed the final solution with pyridine to a concentration of about 40 per cent in order to stabilize the color. Using the method in routine clinical work, we have found that the pyridines from different factories give very different color strengths to the final solution of the same amount of acid. Even when the pyridine was distilled at a low temperature, its stabilizing ability soon grew weaker, and the color strength decreased. Only one of the tested pyridines did not change in this way. Moreover, the extinction coefficient of the pyridine mixture was not always directly proportional to the citric acid content of the solution. Sometimes a small amount of citric acid, 20 γ or less, did not give a higher extinction than the blank. Finally, the extinction coefficients plotted against the citric acid amounts did not form a straight line, as can be seen from the values of the

*From the Medico-Chemical Laboratory of the Medical Department, Karolinska Sjukhuset (The Carolinian Hospital), Stockholm.
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extinction coefficients divided by the amount of acid in Table I. On the other hand, the color once developed is very stable in pyridine solution (Table II).

TABLE I

Extinction coefficient (E) of varying concentrations of citric acid, and the proportion between this coefficient and the concentration (E/mg.). In the last column correction has been made for the amount of acid which did not give any visible reaction.

γ CITRIC ACID	GLYCEROL TWICE DISTILLED		PYRIDINE			
			REDISTILLED		PURISSIMUM	
	E	E/mg.	E	E/mg.	E	E/mg.
0	0		0		(−0.030)	
50	0.070	1.40	0.040	0.80	0.040	1.40
100	0.145	1.45	0.095	0.95	0.110	1.40
200	0.290	1.45	0.180	0.90	0.250	1.40
300	Too dark		0.360	1.20	0.390	1.40

In order to find a better color-stabilizing substance a great number of solvents have been tried as substitutes for the pyridine. The best one proved to be pure glycerol (glycerinum bidestillatum). When this solvent is used, the color is much stronger than that obtained with the best pyridine, and the extinction coefficient is directly proportional to the concentration of the acid (Table I). On the other hand, the color was not absolutely stable, and started slowly fading after two hours (Table II). This, however, is of no practical consequence. Glycerol of commercial quality may be used, but it gives a somewhat weaker color (Table II).

TABLE II

Extinction coefficient of the solution from 100 γ citric acid made up to 10 ml. read for different periods after the preparation.

MINUTES	GLYCEROL	PYRIDINE	
	TWICE DISTILLED	COMMERCIAL	REDISTILLED
0	0.150	0.130	0.095
60	0.150	0.130	0.095
120	0.150	0.130	0.095
240	0.145	0.120	0.095
300	0.130	0.110	0.095
390	0.120	0.100	0.095

Among the other substances tested, acetone and saturated sodium chloride solution had some stabilizing effect, but they were much inferior to glycerol.

In the experiments of which the results are given in the tables the pentabromoacetone was extracted from its solution in petroleum ether three times, with 3, 2, and 1 ml., respectively, of sodium sulfide solution, according to Pucher, Sherman, and Vickery. The sulfide extracts were transferred to 10 ml. mottles containing 4 ml. of the stabilizing solvent. The solution was made up to volume with the same solvent and read with the light filter S:47 (which was found more convenient than S:43, the filter used by Pucher, Sherman, and Vickery).

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AN AID IN THE DITHIZONE METHOD: A MECHANICAL SHAKER FOR SEPARATORY FUNNELS*

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ONE of the most frequent operations in the determination of lead by the mixed-color dithizone method, as used in this laboratory,[1, 2] is the shaking of the separatory funnels for the purpose of extracting various components from one phase or the other of the mixture. Although each shaking period is only a minute in length, this operation is repeated at least four additional times during the course of each determination. Experience has shown that in making thirty analyses during a day, more than three hours are consumed in shaking when this is done by hand. The tendency is to decrease either the speed or the intensity of shaking when the operator becomes tired.

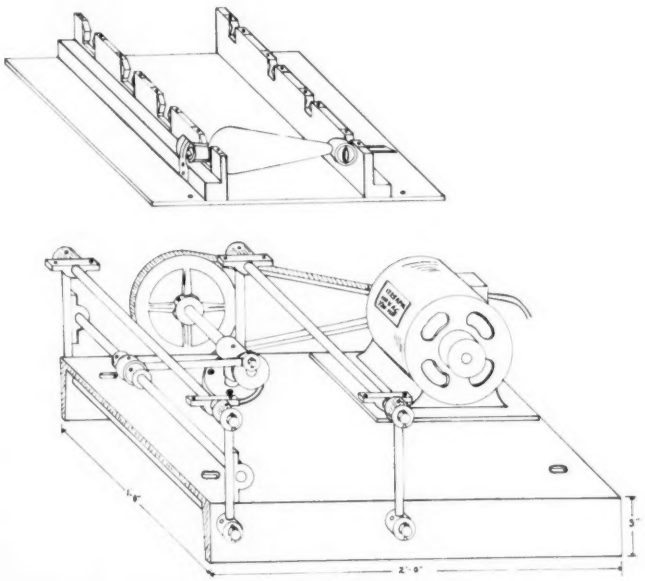

Fig. 1.

In order to expedite the analyses and to obtain uniformly vigorous shaking a mechanical aid was devised (Fig. 1). This consists essentially of a maple rack to hold four 250 ml. Squibb type pyrex glass separatory funnels, this rack being given a reciprocating motion by means of an eccentric driven by a $\frac{1}{20}$ H.P. electric motor 1,725 r.p.m. The figure indicates the component parts of the device, with the rack removed to show details of the driving mechanism. The

*From the Division of Industrial Hygiene, National Institute of Health, United States Public Health Service, Federal Security Agency.
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connecting rod crankpin is offset $\frac{9}{16}$ inch from center of eccentric, and the center-to-center length is $7\frac{1}{4}$ inches for the connecting rod and $6\frac{5}{8}$ inches for the four rocker arms. The heavy section of channel iron is bolted to a large box (18 by 28 by 20 inches high) filled with sand and mounted on No. 14 rubber stoppers to absorb the vibration.

The most difficult task is to hold the funnels securely, since they vary not only in length but also in the outside diameter of neck and stem as well as in length of neck and stopper. Of the several arrangements tried, the one described is the most satisfactory. Phosphor-bronze spring stock was bent to form clips as shown, so that after pushing the funnel down past the middle of the slot there is no tendency for the funnel to loosen up during shaking. The distance between the two slotted boards is slightly greater than the longest funnel in use. The spring holding the stopper in place has sufficient tension to prevent the stopper from coming out and allows for any differences in length of neck. By this arrangement no metal comes in contact with either funnel opening, thus avoiding contamination.

The funnels can be rapidly inserted and removed from the holders using only one hand. With pulleys 1 inch and 6 inches in diameter about 350 double oscillations are produced per minute. With an electric timing switch added, the minute occupied in shaking the four funnels can be used to carry out other operations. Two of these shakers, constructed in this laboratory, have given satisfactory service for over two years.

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MEDICAL ILLUSTRATION

WATER COLOR DRAWINGS FOR SCREEN PROJECTION

FRANCES SHULTZ, BALTIMORE, MD.

HERETOFORE a transparent base has not been used extensively for hand-drawn pictures in color and tone. Such water color illustrations are made to serve as lantern slides primarily, but in the past color in flat washes or lines was used. Color also has been applied over photographs in tone, thereby producing the tonal effect. By experimenting over a period of six months, a method has been found for making a picture that is smooth enough to be projected on a sizable screen for lecture purposes. Of course, since color photography has taken such an important place in the illustrative field, the scope of the subject material for hand-colored lantern slides has been reduced. However, the hand-made slide still serves those items which cannot be photographed as clearly as they can be drawn, such as peritoneoscopic, bronchoscopic, and cystoscopic subjects.

When a transparent medium first was considered, several different materials presented themselves as surfaces upon which to work, for example, glass, cellophane, cellulose, film and other available transparent materials. I tried putting a clear varnish, similar to the one used by artists, on cellophane to keep it from wrinkling, but it became streaked when dry. It was then put on glass, but the same result was obtained. Ammonium hydroxide washed upon blue-tinted roentgenographic film and allowed to dry worked fairly well so far as shading of the different parts was concerned, but it changed some of the colors, for instance, blue became green.

About this time an article written by Dr. M. Muschat on "A New Technique in Medical Illustration" appeared in this JOURNAL. The article dealt with colored illustrations for lantern slides and was accompanied by a black-and-white print of a colored drawing by the author which did not seem to be smooth enough for projection. I was able to obtain some of the material he used, and on examination found it to be similar to other cellulose acetate and cellulose nitrate film, except that it had a ground surface on one side that was excellent to draw upon in pencil. When water colors were applied, however, they became slightly gray and could not be worked to a smooth finish. In short, the film was difficult to handle. Numerous experiments were made to determine which solution and which material would work together to produce this smooth finish. A bit of liquid glue in a small amount of water works well when applied to the film, but after this solution dries it cannot be softened sufficiently to scratch off the high lights without spoiling the film underneath. However, it is an aid in putting on a smooth tone and should not be discarded entirely.

The best method to use in making a smooth lantern slide is to prepare a solution of one part of gum arabic crystals to eight or ten parts of water. This may be thinned if desired. Paint the whole surface of a piece of blue-tinted roentgenographic film from which the gelatin surface has been removed by soaking it for a few minutes in warm water, and allowing it to try. The water colors can then be used in the ordinary way. When the colors are dry and you are ready to scratch off the high lights, dampen the end of an etching tool which has been sharpened to a fine point and carefully scratch off the paint and the solution. The film itself will show through the solution and give the appearance of the highest high light. Insert the film upon which your drawing has been placed between two lantern slide cover glasses and bind with lantern slide binding tape. If you wish, you may make a mat of black opaque paper for the drawing and put it between the two cover glasses before they are bound.

Another medium upon which such colored drawings can be made is the cleared but unexposed and undeveloped lantern slide plate. For example, take a new lantern slide plate from the pack and place it in a photographic fixing bath. This bath clears the plate and leaves a piece of glass with a transparent film base. Such a base supplies the "tooth" to hold the water color pigments which should be applied in thin washes. It is the custom of medical illustrators to scratch or to engrave high lights out of their drawing base or surface rather than to apply white or light pigments with a brush. Because the lantern slide is a transparency, it is essential that the high lights be engraved out rather than built up. In applying the water colors to the surface as described here, the surface becomes damp and, therefore, unsuitable for engraving out the high lights until it is thoroughly dry. Once it is dry, it answers the purpose satisfactorily.

Many water-color pigments have an opaque base and are entirely unsuitable for the coloring of lantern slides and similar transparencies. For instance, the true earth pigments, such as yellow ocher and raw sienna, are particularly opaque and therefore unsuitable. All whites are opaque in varying degrees. For this reason, truly transparent colors should be used. These colors include some of the aniline dyes, which are invariably synthetic derivatives. Most of the dyes used in staining microscopic slides are of the transparent variety, but all of these do not lend themselves to the coloring of transparencies owing to their lack of permanence. The degree of permanence can be ascertained from a majority of the reputable manufacturers of colors. A fair degree of permanence is essential because the rays of light passing through a lantern slide are so great or intense at the point where the slide is placed in the machine that fugitive pigments will change rapidly. This change is caused by the light and heat intensity produced by the average projection bulb. The purchaser can do little more than rely on the word of the pigment manufacturer as to the permanence of his product. However, the transparency of a pigment can be tested by observing a small amount of it under a microscope with transmitted light. By this method the tiny particles of a pigment which appears to be of a definite color by reflected light become black or gray by transmitted

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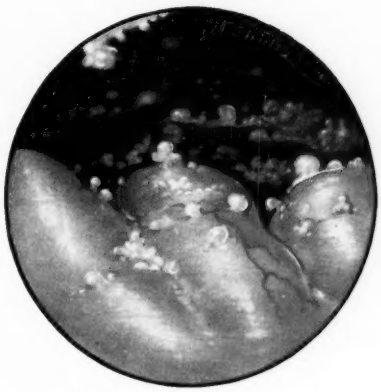
Tubo-ovarian abscess.



Tubercular lesion of the colon and abdominal wall.

These are typical examples of the method of illustration described in the text.

light. Genuine transparent pigments retain their color when studied by transmitted light. The artist must, therefore, choose a palette composed of transparent colors if he is to color lantern slides. Even if one opaque pigment gets mixed in with the rest of the palette a lack of transparency and brilliance will result. In art this is termed *muddiness* or *muddy color*.

A set of transparent water colors may be bought from most art and photographic supply houses in book or liquid form. These serve well for the purpose.

As a rule, it is best to dampen the working surface before the colors are applied. A pencil sketch on paper may be placed beneath the transparent material to serve as a guide in applying the color.

It must be remembered that any flaw, particularly a dark one, will show up when the slide is projected. Therefore, it is advisable to make the picture with great care. I use a stamp collector's magnifying glass, which has a swivel neck and a stand, through which to work. It aids materially in making such illustrations.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCLE BACILLI, Detection of, by Fluorescence Microscopy, Bogen, E. Am. Rev. Tuberc. **44**: 267, 1941.

Sputum or other material, preferably after concentration, is thinly smeared on a glass slide. The slides may then be stained with any acid-fast staining technique, except that the carbolfuchsin is replaced by auramine. The staining solution is made by placing 1 Gm. of pure auramine powder in a flask, gradually adding 1,000 c.c. of distilled water, shaking strongly until it is completely dissolved, and then adding 50 c.c. of pure, liquefied phenol, and again shaking. A temporary clouding later settles out, leaving a clear yellow solution which keeps for a long time in the dark. Hagemann recommends staining with auramine for fifteen minutes at room temperature, and decolorizing with acid alcohol without counterstaining.

The following procedure, after Herrmann, has been used at the Olive View Sanatorium:

1. Fix with heat.
2. Flood with auramine solution, and steam for five minutes.
3. Wash in running water.
4. Decolorize in 70 per cent alcohol, containing 3 per cent hydrochloric acid, until the preparation is colorless.
5. Wash in running water.
6. Dip in a 0.1 per cent aqueous solution of potassium permanganate (this solution remains usable for about a week after turning brown).
7. Wash in running water.
8. Dip in Löffler's alkaline methylene blue solution.
9. Wash in running water.
10. Dry and examine with the fluorescence microscope.

Fluorescence microscopy is a new method for detecting tubercle bacilli. Its theoretical basis suggests advantages over the present microscopic procedures, both in speed and delicacy. An intense violet or ultraviolet source of radiation is required. The bacilli are stained with the fluorescent dye, auramine, and examined with an ordinary microscope equipped with special light filters. Practical experience confirms its predicted value. In 1,000 duplicate smears stained in parallel by the Ziehl-Neelsen and fluorescence methods, there were over 20 per cent more positive tests by the newer technique. In 250 parallel cultural and fluorescence microscopic examinations, the specificity of the latter was confirmed, although the cultural methods still gave considerably more positive findings.

JAUNDICE, Significance of Rise of Non Protein Nitrogen in, Meyer, K. A., Popper, H., and Steigmann, F. J. A. M. A. **117**: 847, 1941.

The nonprotein nitrogen of the blood is increased in patients with both medical and surgical jaundice of severe involvement. The increase is more pronounced in cases in which there is a fatal outcome.

The determination of the nonprotein nitrogen is of diagnostic, and especially prognostic, value in such cases, since it points to an aggravation of a parenchymatous jaundice and to the appearance of a secondary liver damage in cases of obstructive jaundice. Increase of nonprotein nitrogen in a case of surgical jaundice suggests an early operative intervention.

The increased nonprotein nitrogen is to some extent explained on the basis of a reduction of glomerular filtration, but to a greater extent is due to increased reabsorption of urea in the kidney tubules.

An increased breakdown of protein as a cause of the increased nonprotein nitrogen is not evident from the authors' experiments.

BACTERIOLOGICAL TECHNIC: Improved Method of Obtaining Bacteriological Specimens From Wounds Treated by the Closed Plaster Method, DeWaal, H. L. Brit. M. J., Aug. 23, p. 268, 1941.

After the wound has been cleaned, sterile thick absorbent thread is laid backward and forward across its surface, starting at the center and passing to the periphery. The free end of the thread lies away from the wound. The whole of the wound surface is now covered with sterile vaseline gauze. The free end of the thread is passed through a "one-piece" vulcanite cylinder with a flat base and with the sharp edges smoothed off. (The vulcanite should be of good quality and able to resist autoclaving.) A groove is cut in the flat base of the cylinder in which the thread lies. This allows for the subsequent easy withdrawal of the thread. A sterile rubber stopper is inserted into the upper opening of the cylinder. While the plaster is being applied, an assistant steadies the cylinder at the edge of the vaselined gauze (about 1.5 cm. from the wound margin). In taking specimens the procedure adopted is as follows:

The rubber stopper is first removed, and with a swab slightly moistened in antiseptic the inside of the cylinder is cleaned and then dried with another swab. That portion of thread which had been within the cylinder and had come in contact with the antiseptic is now withdrawn, cut off, and discarded. A further piece of the thread is pulled out (about 1.5 cm.), snipped off, and placed in a sterile test tube. This portion of the thread has been exposed to air, and should be examined as a control. The next few centimeters are similarly collected, and give a representative sample of the wound flora.

Depending on the length of the thread originally laid on the wound, five or more specimens may be taken at varying intervals. The last portion of the thread comes from the center of the wound, the part likely to heal last. After each specimen is taken, a fresh sterile rubber stopper is inserted in the mouth of the cylinder.

LYMPHOCYTOSIS, Infectious, Smith, C. H. Am. J. Dis. Child. 62: 231, 1941.

Two types of lymphocytic blood reactions are described to which the term infectious lymphocytosis has been applied.

In one type, which occurs infrequently, unexpected hyperleucocytosis with absolute and relative lymphocytosis appears for a short period and is not accompanied by any recognizable symptoms or physical signs. In the two cases of this type reported in this paper the maximum white blood cell counts were 44,300 and 98,000 cells per cubic millimeter, respectively.

The second type is frequently encountered in pediatric practice and includes cases in which after an infection of the upper respiratory tract low-grade fever continues to be present for prolonged periods. Associated symptoms include anorexia, pallor, fatigability, and para-umbilical pain. The blood in such cases shows a lymphocytic preponderance instead of the expected neutrophilic response and moderate leucocytosis. This symptom complex occurs most characteristically in infants and in young children.

Examination of bone marrow in three cases showed an increase in lymphocytes as the only abnormality.

These two types are classified as acute and chronic infectious lymphocytosis, respectively, and are differentiated clinically, hematologically, and serologically from infectious mononucleosis, leucemia, and miscellaneous infections associated with lymphocytosis.

It is pointed out that many lymphocytic blood responses in children whose titer of heterophilic antibody is normal belong in the class of infectious lymphocytosis rather than infectious mononucleosis.

Acute and chronic infectious lymphocytosis may represent separate entities, although there is some evidence that they may be related.

The etiologic agent of each type is probably not represented by the bacterial flora obtained from the nasopharynx, but probably is an as yet undetermined virus related to infection of the upper respiratory tract.

The prognosis in both types of infectious lymphocytosis is favorable. Treatment of the chronic type is symptomatic and should be directed toward relieving nasopharyngeal infection.

CHICKENPOX, The Blood Picture in, Holbrook, A. A. Arch. Int. Med. 68: 294, 1941.

This study was made during an epidemic of chickenpox in Milwaukee County in the fall of 1939. The 56 patients and 15 controls were all quarantined in their homes.

The following significant tendencies are noted in the patients:

1. The total white blood cell count falls during the end of the incubation period, reaches the lowest level on the second and third days of the rash, and then rises to its former level.

2. During the leucopenia the percentage of polymorphonuclear cells falls, and the percentage of lymphocytes rises, as lymphoblasts and prolymphocytes come into prominence. Among the latter, the mitotic figures, which are occasionally seen, attest further to the strong stimulus to lymphocytic activity which apparently accompanies chickenpox.

3. An increase in plasma cells is also noteworthy early in the eruption stage, their portion of the total differential count sometimes reaching 6 to 8 per cent.

4. In some instances, several of the foregoing features may appear before the rash and thus may be of diagnostic value.

The studies on the control group show the following tendencies:

1. In the aggregate, the various plotted curves for total white blood cell counts and their fractions tend toward a horizontal line.

2. In certain individual cases, a fall in the percentage of polymorphonuclear cells and a rise in the percentage of lymphocytes, with prolymphocytes increasing, occur at about the time the eruption is expected.

VITAMIN C and Serum Protein Levels in Wound Disruption, Hartzell, J. B., Winfield, J. M., and Irvin, J. L. J. A. M. A. 116: 669, 1941.

The authors would emphasize Mason's statement that "the urge to heal is almost as great as the urge to live." Wound healing is affected by many factors, and the nutritional condition of a patient is a most important one. Either to maintain life or to repair tissue there must be a supply of proteins, fats, carbohydrates, minerals, water, and vitamins. Periods of inanition or faulty nutrition are likely to deplete the carbohydrates and fat stores and to lower serum protein and vitamin levels. The authors almost universally supply carbohydrate and salts by routine intravenous administrations, but, generally speaking, if a transfusion is given, it is because the hemoglobin and the red blood cell count are low rather than because the serum protein is low, and the vitamins are forgotten entirely. It would seem logical that, if normal healing is to be expected, the ascorbic acid and serum protein levels of the blood plasma should be brought to normal concentration and kept there.

MENINGOCOCCI AND GONOCOCCI, Simple Method for the Isolation and Identification of, Boisvert, P. L., and Fousek, M. D. Am. J. Dis. Child. 61: 710, 1941.

Twenty-six strains of meningococci and 8 strains of gonococci were isolated in the pediatric laboratory during a three-year period; the carbon dioxide jar of Thompson was used for the cultures. The organisms grew out well in twenty-four hours on blood agar plates and were recovered from material from all infected patients on the first culture. It was found that the simple addition of the differentiating carbohydrates to the blood agar afforded a satisfactory method for further identification of the organisms by fermentation tests.

Media.—A fresh beef heart infusion with 1 per cent neopeptone was the basic medium used. The blood agar plates used for the isolation of the organisms were made by adding 10 c.c. of defibrinated rabbit's blood to a flask containing 200 c.c. of melted infusion agar. The blood and agar were mixed, and the mixture was poured into sterile Petri dishes and allowed to solidify. The contents of one flask made about fifteen blood agar plates.

For the fermentation tests blood agar plates containing dextrose, maltose, and levulose were used. Five-tenths cubic centimeter of defibrinated rabbit's blood was added to a tube containing 10 c.c. of melted infusion agar. The contents of the tube were mixed and

poured into a sterile Petri dish containing 2 c.c. of 10 per cent dextrose. An even distribution of blood agar and carbohydrate was obtained by gently tilting the dish back and forth while the contents were still liquid. Blood agar plates with maltose and levulose were made in the same manner.

Carbon Dioxide Jar.—The apparatus consisted of a round, flat-bottomed 2.5 liter pyrex jar, a square cover with corked aperture, and a container for reagents. It differed only in minor details from the apparatus used by Thompson. The container for reagents was fastened to the bottom of the jar with sealing wax. Petrolatum was used to seal the cover to the jar.

Sodium bicarbonate and sulfuric acid were the reagents used. The sodium bicarbonate solution was made by dissolving 84 Gm. of sodium bicarbonate in 1 liter of water. The sulfuric acid solution contained 20 c.c. of concentrated sulfuric acid per 580 c.c. of water. Equal amounts of each reagent were used, and 22.4 c.c. of carbon dioxide were formed from each 1 c.c. of sodium bicarbonate solution. An amount of each solution, equal to 0.45 per cent of the volume of the jar, furnished an atmosphere of about 10 per cent carbon dioxide.

Isolation of Strains.—Purulent discharge from the eyes or the genital tract was obtained on a sterile cotton-tipped wooden applicator. It was smeared over a small area near the edge of a blood agar plate and streaked with a platinum loop.

A 0.1 c.c. sample of spinal fluid was placed on a blood agar plate with a sterile pipette and streaked in a similar manner.

Blood cultures were made by adding 5 to 7 c.c. of the patient's blood to 50 c.c. of infusion broth and by mixing 2 c.c. of the citrated blood of the patient with 10 c.c. of infusion agar to form a pour plate.

Material for culture was obtained from the nose by introducing a cotton-tipped wooden applicator into the nostrils until the nasopharyngeal wall was reached; material from the throat was secured by touching both tonsils and the posterior portion of the pharynx. The material from the nose and that from the throat were streaked separately on blood agar plates.

The inoculated media were put into the carbon dioxide jar, the calculated amount of sodium bicarbonate solution was placed in the container for reagents, and the jar was covered. An amount of sulfuric acid equal to that of the sodium bicarbonate solution was added through the hole in the cover by means of a pipette. The hole was closed with a cork, and the jar with its contents was incubated overnight at 37.5° C. Portions of colonies on the plates which resembled meningococci or gonococci were smeared on a glass slide, stained, and examined microscopically. Blood cultures positive for these organisms were examined in a similar manner. If gram-negative diplococci were found, the organisms were further identified by their fermentation reactions.

Fermentation Tests.—Three blood agar plates, containing dextrose, maltose, and levulose, respectively, were inoculated and incubated overnight in the carbon dioxide jar. One drop of 2 per cent bromthymol blue was placed on each of the plates in an area where colonies were present. Fermentation of the sugar was indicated by a change in the color of the indicator from dark blue to yellow.

BLOOD SERUM, Desiccated, Viability of the Spirochetes of Syphilis and Yaws in, Turner, T. B., Bauer, J. H., and Kluth, F. C. Am. J. Med. Sc. 202: 416, 1941.

T. pallidum and probably *T. pertenue* are commonly killed by the process of freezing and desiccation, even when the method is such that the viability of many other bacteria and viruses is retained. Transfusion of desiccated blood serum or plasma is, therefore, probably without risk as regards the transmission of syphilis or yaws, even though the material is obtained from an infected donor.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

Genital Functions and Their Hormonal Regulation*

INTERNATIONALLY known for his work in endocrinology, Dr. Zondek in this monograph presents and discusses the results of investigations carried on in the Hebrew University and the University Hospital in Jerusalem.

In six main sections are discussed: I. The Occurrence of Some Estrogenic Substances in Nature; II. Experimental and Clinical Investigations of the Percutaneous Use of Estrogenic and Androgenic Hormones; III. The Effect of Protracted Treatment With High Doses of Estrogenic Hormone; IV. On the Fate of Estrogenic Hormones in the Organism; V. Clinical Investigations Concerning the Cycle of the Female Organism; VI. The Mechanism of Menstruation.

Of interest is the report in the opening section of the demonstration of estrogenic hormone and a flavin derivative (vitamin B_2) in the water and sandy slime of the Dead Sea.

In Section III Dr. Zondek reports absence of any evidence that protracted hormone therapy may be, as has been suggested, a factor in carcinogenesis.

Space does not permit any extended résumé, but it can be said that these studies are of great interest and value to the investigator in this field and to the clinician as well because of their clinical aspects.

Well printed and excellently illustrated, this is a valuable contribution. A summary completes each chapter and a bibliography is appended.

Essentials of Endocrinology†

AS THE author comments in his preface, the field of endocrinology is marked at the present time by "feverish activity." His book, therefore, will be welcomed by those who wish to have at least a bird's-eye view of what has now become, in many respects, a fundamental science.

In this book Dr. Grollman presents a well-balanced survey of the present status of endocrinology and discusses in seriatim: Endocrine Glands of the Cranial Cavity; The Bronchiogenic Organs; Endocrine Glands of the Abdominal Cavity; Hormones of the Reproductive System; and The Hormones Derived From the Nonendocrine Glands.

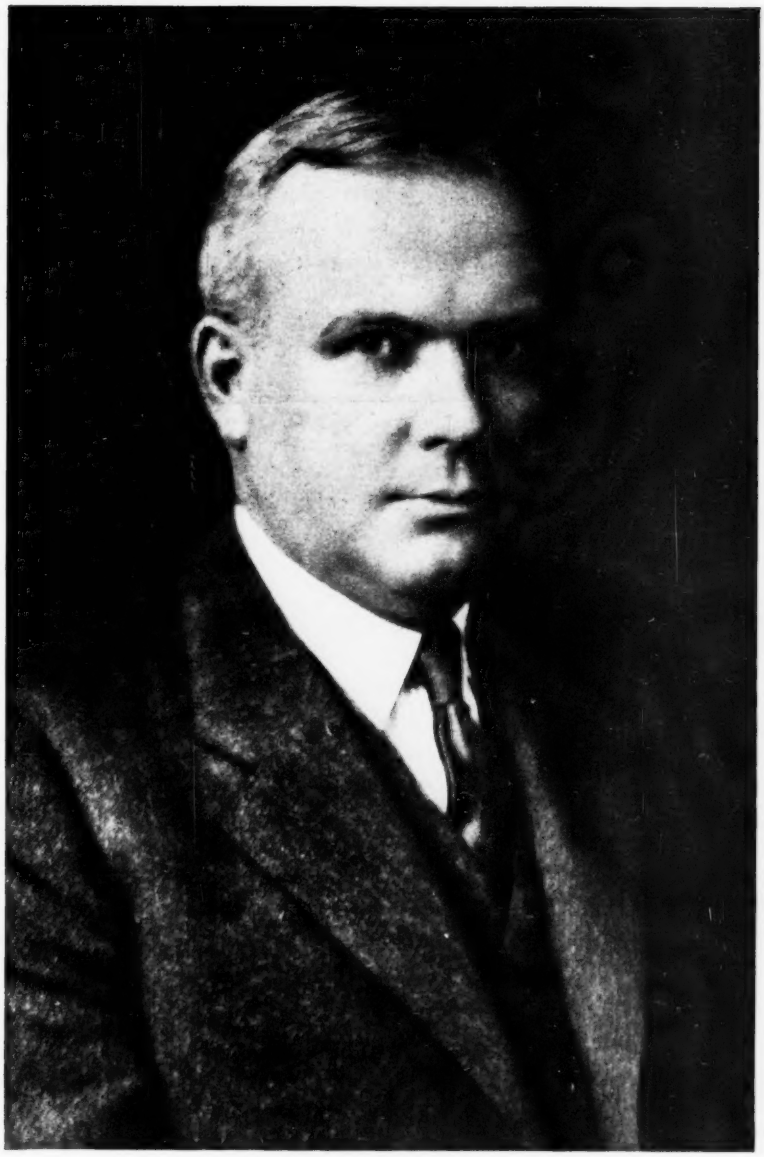
A useful reference list of available endocrine products is found on the cover pages, front and back.

Well oriented and organized, well written and illustrated, this is a useful book deserving of wide circulation.

*Clinical and Experimental Investigations on the Genital Function and Their Hormonal Regulation. By Bernhard Zondek. Cloth, 264 pages, 59 figures, $4.50. Williams & Wilkins Co., Baltimore, Md.

†Essentials of Endocrinology. By Arthur Grollman, Ph.D., M.D., Associate Professor of Pharmacology and Experimental Therapeutics, Johns Hopkins University, Medical School. Cloth, 480 pages, 74 illustrations, $6.00. J. B. Lippincott Co., Philadelphia.

In Memoriam



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1874-1941

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